

**Selected papers from the International Conference
'A century of forest knowledge – education, innovations,
challenges' (CENFORKNOW 2025), Sofia, Bulgaria**

First of two special issues featuring peer-reviewed papers from CENFORKNOW 2025

This volume of *Forestry Ideas* (vol. 31, No Special Issue 1) presents selected peer-reviewed papers from the International Conference 'A century of forest knowledge – education, innovations, challenges' (CENFORKNOW 2025), held in Sofia, Bulgaria – 8–10 May 2025. The issue is dedicated to contemporary research and innovations in the science and practice of natural resource management and stewardship, as presented and discussed during the conference sessions.

Due to the large number of high-quality contributions received, a second special issue (Special Issue 2) will follow, containing additional peer-reviewed papers from the same event. Together, these two issues aim to offer a comprehensive representation of the scientific output of CENFORKNOW 2025.

All articles in this Special Issue follow the journal's standard editorial and peer-review policies.

Guest Editors' Note

We are pleased to present this first of two special issues of *Forestry Ideas* (vol. 31).

This Special Issue has been made possible thanks to the joint editorial efforts of the following Guest Editors:

- Assoc. Prof. Dr. Stoyan Stoyanov – Faculty of Forestry, University of Forestry in Sofia
- Prof. Dr. Rumen Tomov – Faculty of Agronomy, University of Forestry in Sofia
- Prof. Dr. Peter Zhelev – Faculty of Forestry, University of Forestry in Sofia
- Assoc. Prof. Dr. Momchil Panayotov – Faculty of Forestry, University of Forestry in Sofia

Each of the Guest Editors has contributed to the selection, coordination, and scientific review of the *manuscripts*, ensuring academic rigor and thematic coherence. Their combined expertise reflects the interdisciplinary nature of the contributions included in this issue.

We extend our appreciation in advance to all authors and reviewers whose work will be featured in No Special Issue 2, which will be published later in 2025.

We sincerely thank all authors and reviewers for their valuable contributions. We hope that this collection will serve as a useful reference for researchers and practitioners and will inspire further advancements in the management and stewardship of natural resources.

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A Century of Forest Knowledge: Reflections from an International Scientific Milestone

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Abstract

This special issue of the Forestry Ideas is dedicated to the international scientific conference ‘A Century of Forest Knowledge: Education, Innovations, Challenges’, held to mark 100 years of university forestry education in Bulgaria. This event served as a significant academic forum for forestry researchers, practitioners, and policymakers. Given the unprecedented pressures facing forest ecosystems – from climate change and extreme weather events to prolonged droughts, wildfires, and other global challenges – the role of forest science and education has become more critical than ever. The conference placed strong emphasis on sustainable forest management, biodiversity conservation, ecosystem restoration, and the protection of old-growth forests. These themes reflect the urgent need for integrated, evidence-based approaches to forest management and conservation. The conference brought together a vibrant international community, featuring 116 scholarly contributions from 433 co-authors across 13 countries and over 30 academic and research institutions. We were also honoured to host participants from scientific organisations, governmental bodies, and non-governmental organisations, further underscoring the interdisciplinary and collaborative spirit that defines modern forestry science. The main thematic fields of the conference were: Climate change and forests; Ecosystem biodiversity, conservation and environmental restoration; Agriculture in mountainous and semi-mountainous regions; Forest disturbances and management challenges; Forest stand dynamics, growth and yield; Forest policy and key challenges; Forest operations and wood processing; Wildlife conservation and management; Landscape architecture.

Key words: biodiversity, conservation, ecology, education, forestry, management.

One hundred years ago, on 28 January 1925, the Academic Council of Sofia University resolved to establish the Department of Forestry within the Faculty of Agronomy, thus laying the foundation for higher forestry education in Bulgaria. Today, we celebrate a century of knowledge dedicated to forests – a century of educa-

tion, innovation, and challenges.

This centennial milestone is a source of well-deserved pride and an occasion for reflection. Over the past 100 years, our faculty has much to celebrate and be proud of: the pioneering professors who shaped a generation of young foresters instrumental in restoring Bulgaria’s forest

wealth; the thousands of alumni who have contributed to forest conservation; and the foresters who, even today, uphold the honour of their profession, faithfully serving Bulgaria's forests and preserving them for future generations. The forests of Bulgaria, as we know them today, owe their existence to the tireless efforts and dedicated service of generations of foresters educated at the University of Forestry.

Forestry emerged as a scientific discipline in Europe, and later took root in Bulgaria, in response to industrialisation, deforestation, soil erosion, and flooding. The need for afforestation, flood control, and the mitigation of natural disasters, which had become evident over the past few centuries, was recognised by Bulgarian society. The first foresters undertook the noble mission of creating new forests, addressing these environmental challenges with commitment and vision.

Nowadays, forestry aims to conserve and naturally regenerate forests, preserve biodiversity and old-growth forests, and maintain their ecological, climate-regulating, water-protective, and recreational functions. The forest wealth of Bulgaria is of inestimable value owing to its ecological functions, diverse species composition, and the presence of primary forests. The country ranks among Europe's leaders in terms of the biodiversity of forest ecosystems and the highest proportion of old-growth forests. A significant part of them is included in the Natura 2000 European ecological network. Forested areas in the country span 4.18 million hectares, accounting for 37 % of its territory. Almost 8 % of the total forested area is protected, 65,688 ha of forests are in reserves of different categories, and 78,359 ha (2 % of the forests) within national parks (Angelstam et al. 2021, Nagel et al. 2025). Old-growth forests designated by Ministry

of Agriculture and Food cover 109,303 ha (2.81 % of the total forested area). Most old-growth forests are located within national parks and reserves. This achievement is a testament to the contributions of our faculty's alumni, as we celebrate the 100th anniversary.

However, the situation was markedly different a century ago. Looking back, we encounter a starkly contrasting picture. At the end of the 19th and the beginning of the 20th century, the condition of Bulgaria's mountains and forests was disastrous. The 'ancient and impenetrable' medieval forests had all but vanished, surviving only in the accounts of travellers. Contemporary evidence of this past grandeur is found in the few remaining majestic, silent forests such as Tulovska Koriya, Marashka Koriya, and Yulievka Koriya (Tsavkov et al. 2025). According to data from Felix Vogely, published in his first manual on 'Torrent Control and Afforestation' (1911, pp. 68–79), 168 floods were recorded along the Maritsa, Yantra, Iskar, and Tundzha rivers in the first 11 years of the 20th century, resulting in 84 human casualties and damage exceeding 1 billion Bulgarian leva (BGN). These devastating floods swept away fertile lands, denuded mountain slopes, and even claimed human lives. From 1900 to 1926, Bulgaria's forest area decreased from 3 million to 2 million hectares, representing a mere 19 % of the country's total area.

In recent decades, Bulgaria's forests face numerous challenges – climate change, prolonged droughts, extreme weather events, floods, natural disasters, unprecedented forest fires, and the decline of some coniferous forests established in the past. Many of these challenges are of global significance. Foresters must address these issues, requiring modern education aligned with current

trends, innovative approaches, and a robust scientific foundation.

Celebrating a century of forest knowledge, the University of Forestry, which includes the Faculty of Forestry, stands as a modern educational and scientific hub. Originating as the successor to the Faculty of Agronomy and Forestry at Sofia University and having operated as an independent higher education institution for 72 years (Fig. 1), the University of Forestry now educates students across five faculties, offering 11 bachelor's, 28 master's, and 36 doctoral programmes. It is not an easy task to summarise 100 years of history in a few pages, but significant and valuable information has been published in several articles and books (see Stoyanov 1968, Milev et al. 2010, Iliev et al. 2023, Tsavkov 2025).

This special issue of the *Forestry Ideas* is dedicated to the international scientific conference 'A Century of Forest Knowledge: Education, Innovations, Challenges',

held to celebrate 100 years of university forestry education in Bulgaria. This event served as a significant academic forum for forestry researchers, practitioners, and policymakers.

Given the unprecedented pressures facing forest ecosystems – from climate change and extreme weather events to prolonged droughts, wildfires, and other global challenges – the role of forest science and education has become more critical than ever. The conference placed strong emphasis on sustainable forest management, biodiversity conservation, ecosystem restoration, and the protection of old-growth forests. These themes reflect the urgent need for integrated, evidence-based approaches to forest management and conservation. Forests support countless rare and endangered species and provide vital environmental and social functions.

In the face of today's dynamic environmental challenges, decision-making

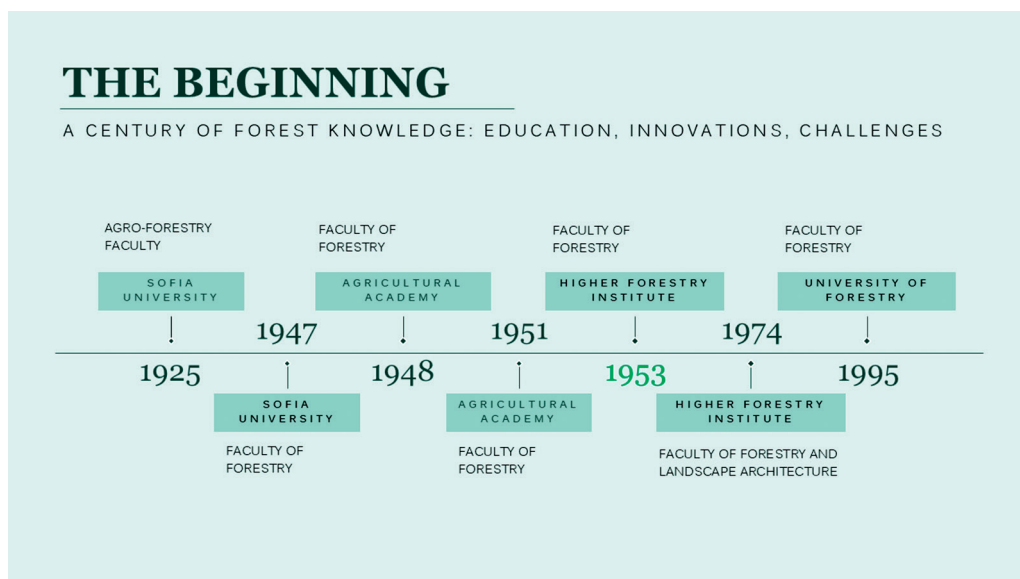


Fig. 1. A century of higher forestry education in Bulgaria: The timeline of the most important milestones in the history of University of Forestry.

in forestry management and conservation is fraught with uncertainty. Climate variability, shifting ecological conditions, and emerging threats such as invasive species and large-scale forest disturbances introduce significant risks. These complexities challenge policymakers and forest managers to make informed decisions with long-term implications. Adaptive resource management has become a cornerstone of modern forestry, enabling flexible, evidence-based strategies that respond to changing conditions. Advanced ecological and forestry models play a critical role in this process, providing predictive tools to assess risks, simulate ecosystem dynamics, and guide sustainable practices. By integrating data-driven models with adaptive management, foresters can better navigate uncertainties, ensuring the resilience of Bulgaria's forests while balancing conservation goals with societal and economic needs. These approaches underscore the importance of continued innovation and interdisciplinary collaboration in shaping effective policies for the future.

The conference brought together a vibrant international community, featuring 116 scholarly contributions from 433 co-authors across 13 countries and over 30 academic and research institutions. We were also honoured to host participants from scientific organisations, governmental bodies, and non-governmental organisations, further underscoring the interdisciplinary and collaborative spirit that defines modern forestry science. The main thematic fields of the conference were: Climate change and forests; Ecosystem biodiversity, conservation and environmental restoration; Agriculture in mountainous and semi-mountainous regions; Forest disturbances and management challenges; Forest stand dynamics,

growth and yield; Forest policy and key challenges; Forest operations and wood processing; Wildlife conservation and management; Landscape architecture.

The articles selected for inclusion in this special issue reflect the depth and diversity of the conference proceedings. Topics range from innovative forest monitoring technologies and climate-adaptive silvicultural practices to policy-oriented research on governance, education, and stakeholder engagement. Taken together, these contributions illuminate recent progress in forest science and delineate key priorities for future research.

The word cloud effectively highlights the main keywords (Fig. 2) from the articles included in the issue, providing a visual representation of the central themes in forestry, ecology, and conservation. The prominence of terms like 'forests', 'management', 'conservation', 'ecology', 'biodiversity', and 'climate' reflects the critical focus on sustainable forest management and the ecological challenges faced today. Other notable keywords, such as 'fires', 'drought', 'carbon', and 'restoration', underscore the pressing issues of climate change, natural disasters, and efforts to mitigate their impacts. The inclusion of terms like 'analysis', 'models', and 'technology' suggests a strong emphasis on data-driven approaches and innovation in addressing these challenges. Additionally, words like 'Bulgaria', 'mountains', and 'European' indicate a regional focus, likely tied to the context of Bulgaria's forest ecosystems and their significance within Europe.

We are confident that the insights presented in this volume will enrich academic discourse and provide useful guidance for both policy and practice. The conference, and by extension this special issue, has fostered valuable international collabora-



wordcloud (Fellows 2018) in *R* (R Core Team 2025).

tion, knowledge exchange, and interdisciplinary dialogue that will continue to shape the next century of forestry science.

We extend our sincere gratitude to all authors, peer reviewers, and members of the editorial and scientific committees for their invaluable contributions to this publication. We also thank the institutions and sponsors who supported the organisation of the conference and the preparation of this special issue.

It is our hope that this collection of work not only marks a historic milestone in Bulgarian forestry education but also serves as a catalyst for continued innovation and cooperation in addressing the complex challenges facing our forests and societies.

As we look to the next century, the University of Forestry remains poised to lead in sustainable natural resource management solutions.

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Estimation of forest fire burned area and burn severity degrees using Sentinel-2 data

Presented at the Conference 'A century of forest knowledge – education, innovations, challenges (CENFORKNOW 2025)', 7–9 May 2025, Sofia, Bulgaria

Thematic field: Forest disturbances and management challenges

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Abstract

A study has been conducted to identify burned areas in the region Otets Paisievo village in Plovdiv District, Bulgaria. The research aims at locating the burned areas and determining the degree of damage caused by a wildfire which occurred in the summer of 2024. Remote sensing and GIS methods were applied for the study. Various spectral indices (NDVI, MIRBI, NBR, NBR2, RBR, BAI, BAIS2) were generated using data from the Sentinel-2 satellite. The separability index (SI) was calculated, which showed the highest values for dMIRBI, dNBR2, dNBR and dBAIS2. Based on these four differential index differences, the burn area was determined and compared with data from the European Forest Fire Information System (EFFIS) and the local and national forestry sector. The results demonstrated a strong correlation with the actual burned area. Based on the analysis of spectral index values and the corresponding histograms, the study area was classified into four burn severity categories: unchanged, low, moderate, and high. The research indicates that for the surveyed area, indices based on shortwave infrared (SWIR) bands are effective in detecting and mapping forest fire burned areas.

Key words: burn area mapping, forest fire, Sentinel-2, separability index, spectral indices.

Introduction

The wildfires occurrence along with the increase in their frequency and intensity lead to the disruption of the essential ecological functions of forests.

The consequences of forest fires have various aspects – ecological, economic,

and social. Providing reliable information about forest areas affected by fires and tracking the processes related to their recovery are of utmost significance for informed decision-making and the implementation of effective forest management measures.

The use of remote sensing technolo-

gies for monitoring large areas from a distance, combined with GIS, will enhance public awareness and improve control over activities in forested areas (Asenova 2023).

In recent years, the availability of high-resolution optical data providing spectral information across different areas of the electromagnetic spectrum has enabled the tracking of environmental processes and performing analyses related to the identification, mapping, and assessment of fire-affected areas.

The use of an ever-increasing range of spectral indices expands the possibilities for distinguishing burned from unburned areas (Tran at al. 2018, Gikov and Dimitrov 2019, Alcaras at al. 2022, Arisanty at al. 2022, Farhadi at al. 2022, Vandansambuu at al. 2023, Guo at al. 2024). Despite the large number of studies related to spectral indices, there is still a challenge to determine which index is most suitable under specific conditions. The difference in fire intensity, type of vegetation, its condition before fire, local characteristics of observed area/region, such as climate, topography, vegetation composition, and initial plant mortality, are all factors that influence the assessment of burned areas and the speed of vegetation recovery (Avetisyan at al. 2022). The behaviour of indices may vary across various ecosystems, suggesting that functionally distinct types of forests have different spectral responses. Therefore, the same index may not perform equally well in detecting burned areas (Mpakairi at al. 2020). As a result, analyses cannot be based on a single, standardised spectral index.

Each index has specific characteristics and applications in the mapping of burned areas. Normalised Difference Vegetation Index (NDVI) is one of the most popular indices, not only for determining vegetation

status and conducting analyses related to susceptibility to erosion, mudflow, and rockslides (Baltakova at al. 2018, Nikolova at al. 2024), but also for assessing the severity of fires. This index uses the ratio of reflectance in the Red and Near-Infrared (NIR) spectral bands and shows that the lower the values of the ratio, the more intense the damage to vegetation is, i.e. the more severe the fire impact is (Kesgin and Ersoy 2020).

Normalised Burn Ratio (NBR) is used to identify burned areas and to assess the severity of burning. It is a ratio of the difference to the sum of reflectance on NIR and SWIR bands. Vegetation before the fire will have a high reflectance value relative to the NIR wavelength and a low reflectance value relative to SWIR wavelength, while the opposite will occur in the post-fire image (Sukmono at al. 2023). In other words, the difference between the spectral responses of healthy vegetation and burned areas peaks in the NIR and SWIR regions of the electromagnetic spectrum. Normalised Burn Ratio 2 (NBR2) modifies the Normalised Burn Ratio (NBR) to highlight water sensitivity in vegetation and may be useful in post-fire recovery studies. NBR2 is calculated as a ratio between SWIR values of Sentinel-2 (B11 and B12), substituting the NIR band used in the NBR with band B11 of the Sentinel-2 (Kesgin and Ersoy 2020). Another widely accepted index used for identifying burned areas, in addition to Normalised Burn Ratio (NBR), is Burn Area Index (BAI). Both indices can be automatically calculated and achieve relatively high accuracy in detecting burned land by means of a simple algebraic algorithm applied to pixels (Wu at al. 2022). It is specifically designed for discriminating burned land in the red-NIR spectral region, highlighting the signal from charcoal in post-fire images. There-

fore, this index is highly dependent on the temporal stability of charcoal after the fire (Chuvieco et al. 2002). Burned Area Index for Sentinel-2 (BAIS2) is a fire detection index specifically designed for the spectral characteristics of Sentinel-2, with its innovation lying in the combination of the red-edge band ratio (used to describe vegetation characteristics) and the response of the short-wave infrared (SWIR) band. This combination demonstrates significant efficiency in identifying burned areas, enabling more precise detection and identification of fire-affected regions (Liu et al. 2025). Relative Burn Ratio (RBR) spectral index is a variant of differential NBR (dNBR), where $dNBR = NBR_{pre} - NBR_{post}$. The RBR index takes into account the relative amount of change before and after the fire by dividing dNBR by pre-fire NBR value. This revitalised index was created to eliminate the bias caused by vegetation type and density before the fire (Chuvieco et al. 1998). Medium Infrared Burn Index (MIRBI) has the ability to differentiate burned areas from bare land (Arisanty et al. 2022). It is designed in the discriminative middle-infrared space, with short and long wavelength bands (referring to Sentinel-2 bands 11 and 12). This

index is highly sensitive to spectral changes caused by burning and relatively insensitive to noise (Trigg and Flasse 2001).

One advantage of using spectral indices is the easy, standardised processing of images, which provides more precise information compared to visual interpretation or automatic classifications (Dimitrov and Gikov 2009).

The main objective of the present study is to determine the burned area and burn severity degrees in the region of the village of Otets Paisievo, using remote sensing and GIS. To achieve this, a methodology has been developed that includes testing various spectral indices and evaluating them, as well as determining optimal threshold values for burned areas and burn severity levels.

Materials and Methods

Study area

The object of the study covers forested areas in the vicinity of the village of Otets Paisievo, located at 40 km from Plovdiv, in the municipality of Kaloyanovo, Southern Bulgaria (Fig. 1).



a)



b)

Fig. 1. Study area: a) Location of the study site – Otets Paisievo village, Plovdiv district, Bulgaria; b) The forest fire in the study area.

The area is hilly, typical of the southern slopes of the Sredna Gora Mountains. The climate is characterised by warm summer, dry autumn, and mild winter. Deciduous and coniferous forests dominate in the surroundings of the village. The main broadleaf tree species are oak (*Quercus* sp.), beech (*Fagus sylvatica* L.), and hornbeam (*Carpinus betulus* L.). The coniferous forests are represented by black pine (*Pinus nigra* J.F.Arnold), Scots pine (*Pinus sylvestris* L.), and spruce (*Picea abies* (L.) H.Karst.), as they are frequently planted in these regions.

On 16 July 2024, a large fire broke out in the village of Otets Paisievo, destroying over 20 houses and forcing the evacuation of 15 residents. The fire affected approximately 900 ha of deciduous and coniferous forests and other areas, such as meadows and pastures (Bulgarian News Agency (BNA) 2024) on the territory in the municipality of Kaloyanovo with available data of burned area in the Executive Forest Agency (EFA) (EFA 2025).

The fire was classified as a top tree

fire, where the flames engulf the treetops, often without visible flames on the ground. Although the terrain was hilly and access was hard, the fire was contained by 18 July 2024. However, smouldering hot-spots remained in some areas and those required monitoring.

Dataset

For this study, data from Sentinel-2 satellite mission provided by the European Space Agency (ESA) (2015) were used. They were downloaded from Copernicus Data Ecosystem website, 2024, the new portal of ESA launched at the beginning of 2023. Sentinel-2 has a set of 13 spectral bands (spectral ranges), covering from the visible and near-infrared to the short-wave infrared bands. It includes four spectral bands with 10 m spatial resolution, six with 20 m, and three with 60 m (Sentinelhub 2025). The specification of Sentinel-2 is shown in Table 1.

Compared to other satellites used for fire localisation, such as Landsat (Gikov

Table 1. Description of the band features of Sentinel-2 images.

Bands	Central wavelength, nm	Bandwidth, nm	Spatial resolution, m
B1 – Coastal aerosol	443	20	60
B2 – Blue	490	65	10
B3 – Green	560	35	10
B4 – Red	665	30	10
B5 – Vegetation Red Edge	705	15	20
B6 – Vegetation Red Edge	740	15	20
B7 – Vegetation Red Edge	783	20	20
B8 – NIR	842	115	10
B8A – Vegetation Red Edge	865	20	20
B9 – Water Vapour	945	20	60
B10 – SWIR – Cirrus	1375	30	60
B11 – SWIR 1	1610	90	20
B12 – SWIR 2	2190	180	20

Source: ESA (2015).

and Dimitrov 2019), Sentinel-2 provides relatively high spatial (Table 1) and temporal resolution (each satellite captures a given area every 10 days; thus, using data from each satellite renders the period between two captures as 5 days).

This study uses data from Sentinel-2, Level-2A as it provides products with atmospherically corrected surface reflectance (SR) from Level-1C products (Gascon et al. 2017, Copernicus 2025). In some studies, corrections related to cloud presence are performed (Gascon et al. 2017), but no such correction was applied in this study because there were no clouds over the study area on the relevant dates.

The images used before the fire date and the closest possible date after it was extinguished are Sentinel-2A, MSIL2A, T35TLH: 08.07.2024 and 28.07.2024.

Methods

The proposed workflow includes the following stages: selection of appropriate images for the purposes of the study, sub-setting, resampling, stacking, generation of spectral indices and their differ-

ence, evaluation of the spectral indices' performance, application of water/cloud mask, determination of precise threshold values using histogram analysis and forestry data, and analysis.

Image processing was carried out in the ArcGIS Pro 3.4.0 software environment (ESRI 2024). A shapefile was created to crop the image dataset so that it would cover only the studied area. The metadata in the generated subsets are linked to the original dataset, ensuring the preservation of essential information from the original image (Stoyanova and Katsarska-Filipova 2023). This step is important as it ensures the analysis of the same area when comparing different time periods and optimises data processing by reducing the data volume and speeding up calculations. One of the subset images is shown in Figure 2.

When calculating indices, such as BAIS2, NBR, etc., bands with different spatial resolutions are used (e.g., B8 – 10 m and B12 – 20 m for NBR), which required them to be standardised to the same resolution. For resampling, the nearest neighbour algorithm was used, which does not alter any of the cell values from the input raster dataset (ESRI 2025).

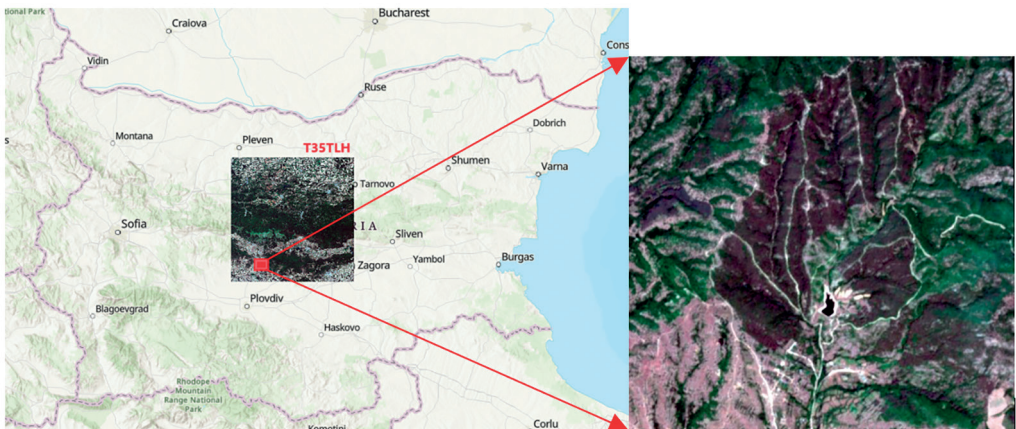


Fig. 2. The entire image on the left and the subset image on the right.

After the pre-processing stage, the creation of spectral indices was carried out. Based on a preliminary review of the topic, a set of characteristic spectral indices were selected for localising burned areas and determining burn severity.

The applicability of seven vegetation indices, both general and specific for identifying burned areas (NDVI, NBR, NBR2, MIRBI, BAI, BAIS2, RBR), were tested for identifying burned zones based on changes in land cover before and after the fire. The spectral indices are listed in Table 2.

A water body was located within the study area, and for this reason, a water mask was created using Normalized Difference Water Index (NDWI). By maximising the reflectance of the water body in the green spectral range and minimising the reflectance of the water body in the

near-infrared spectral range, NDWI index is particularly effective in distinguishing open water areas from other types of land cover, such as vegetation and bare soil. The index values range from -1 to +1, with higher positive values indicating the presence of water (McFeeters 2013, Stoyanova and Katsarska-Filipova 2023). This approach enhanced the reliability of the information in the affected area.

For the accurate calculation of burned areas, determining the threshold value of the index is of critical importance. This threshold defines the point above which pixels are considered to belong to burned areas and below which they are classified as unburned. There is no standardised method for defining burn severity degrees (thresholds).

For example, one widely used scale

Table 2. Description of the spectral indices used in this study adapted for Sentinel-2.

Spectral indices	Algorithm	References
NDVI	$NDVI = \frac{B8 - B4}{B8 + B4}$	Tucker 1979
NBR	$BBR = \frac{B8 - B12}{B8 + B12}$	Key and Benson 1999
Differential NBR	$dNBR = NBR_{pre} - NBR_{post}$	
NBR2	$NBR2 = \frac{B11 - B12}{B11 + B12}$	Key and Benson 2006
BAI	$BAI = \frac{1}{(0.1 - B4)^2 + (0.06 - B8)}$	Chuvieco et al. 1998
BAIS2	$BAIS2 = \left(1 - \sqrt{\frac{B6 \cdot B7 \cdot B8A}{B4}} \right) \cdot \left(\frac{B12 - B8A}{\sqrt{B12 + B8A}} + 1 \right)$	Filipponi 2018
MIRBI	$MIRBI = 10 \cdot B11 - 9.8 \cdot B12 + 2$	Trigg and Flasse 2001
RBR	$RBR = \frac{dNBR}{\sqrt{ NBR_{pre} } - 1}$	Parks et al. 2014
NDWI	$NDWI = \frac{B3 - B8}{B3 + B8}$	McFeeters 1996

for assessing burn severity using the dNBR index is the USGS FireMon scale. It differentiates five classes of areas: unburned, low severity, low to moderate severity, moderate to high severity, and high severity (Key and Benson 2006). In some cases, the two moderate severity classes are combined.

For this study, burn severity is classified into four classes: unburnt/unchanged, low severity, moderate severity, and high severity (Dimitrov and Gikov 2009). These severity levels correspond to the data on the official website of the Ministry of Agriculture and Food by the Executive Forestry Agency in Bulgaria:

- Low severity (25 %) – These are areas with light burn severity, where part of the vegetation has survived. Trees may have scorched branches, but the soil remains relatively unaffected. Rapid ecosystem recovery is expected.

- Moderate severity (50 %) – These are moderately affected areas where approximately half of the vegetation has been destroyed. Some trees may be scorched, but surviving vegetation is still present. Recovery will take longer and may require additional restoration efforts.

- High severity (100 %) – These are completely burnt areas. All trees, shrubs, and grasses have been destroyed, and the soil may be affected by high temperatures. A long-term restoration strategy is needed, including re-forestation and soil rehabilitation.

To determine the precise threshold values (i.e. to avoid marking pixels outside the actual burned area), field data from the local Forest Service were used for validation. These data were collected using mobile GPS devices. However, some areas of the territory are difficult to access and no measurements have been made in them, and the burn severity has been de-

termined indirectly (visually). Therefore, the MIRBI threshold map and the field data have been compared visually. Furthermore, an analysis of the histograms of the individual indices was conducted using natural breaks, enabling a more precise determination of the threshold values. The separability index (SI) is widely used to evaluate the performance of spectral indices in terms of their ability to discriminate burned and unburned areas (Kesgin and Ersoy 2020, Farhadi et al. 2022, Pacheco et al. 2023, Afifah and Astuti 2025). Therefore, it is also used in the present study. It is calculated for each image before and after the fire using equation (1).

$$SI = \frac{|\mu_b - \mu_{ub}|}{\sigma_b + \sigma_{ub}}, \quad (1)$$

where μ_b and μ_{ub} are the average, and σ_b and σ_{ub} are the standard deviations of the samples from the burnt and unburnt areas.

Higher SI values indicate that the given spectral index has a strong separability attribute, whereas lower values reflect that the discrimination of burned and unburned pixels are not as good as the higher SI values. Therefore, $SI > 1$ depicts fine separation, whereas values of $SI < 1$ represent poor separation (Mthembu and Marwala 2008).

Results and Discussion

Burn area delimitation and fire severity

Based on the obtained separability index results, presented in Table 3, it can be stated that the best performance in distinguishing between burned and unburned areas is shown by the MIRBI spectral index, followed by NBR2. Similar results were also obtained in another study with a similar focus (Pacheco et al. 2023).

Table 3. Separability index and burned threshold for various indices.

Spectral index	SI	Burned threshold
NDVI	1.2985	dNDVI ≥ 0.074
NBR	1.7168	dNBR ≥ 0.108
NBR2	2.2572	dNBR2 ≥ 0.036
BAI	3 ⁻⁸	dBAI $\leq -1.791e^{-08}$
BAIS2	1.7538	dBAIS2 ≤ -0.360
MIRBI	2.4263	dMIRBI ≥ 0.280
RBR	0.6013	dRBR ≤ -0.327

Similar close results were also shown by the indices BAIS2 and NBR. These were followed by the NDVI index. The separability indices for RBR and BAI were significantly lower, respectively, indicating lower performance in distinguishing between burned and unburned areas compared to the indices described above.

The dMIRBI spectral index had the highest discriminative ability, whereas the dBAI and dRBR indices showed the lowest performance. Although RBR demonstrated good results for distinguishing burn severity in other studies (Sobrino et al. 2024), in our case, the results were low. This may be due to the presence of agricultural plots of land in the southern part of the study area. In literature, the dBAI index is noted for its strong ability to detect recently burned areas, particularly where the vegetation is predominantly woody and the charcoal rate is high (Chuvieco et al. 2002). Similarly, low results for this index were reported in another study (Kesgin and Ersoy 2020). This is likely because, as in our case, a large portion of the study area contains a mixed presence

of woody and herbaceous vegetation (Scheepers et al. 2014). Accordingly, the results indicate that dNDVI also demonstrates a strong ability to differentiate between burned and unburned areas. Nevertheless, the study focused on those with the highest values.

Although the separability index values of dBAI and dRBR are the lowest, this does not mean that they are unable to distinguish burned areas. Rather, for the studied area, they show greater errors compared to the other indices.

Further evaluation of the capabilities of spectral indices, the application of water masks/cloud masks when necessary, as well as conducting histogram analysis combined with the use of field data, leads to an increase in the reliability of the obtained results. Table 4 presents precise threshold values for the indices in the study.

For all four indices, it was found that the smallest portion of the burned area is characterised by high burn severity, followed by moderate severity, while the largest portion corresponds to areas with low burn severity. According to the dMIRBI values, the distribution of burned areas and their percentage is as follows: high burn severity – 18.9 % (119.44 ha), moderate severity – 28.6 % (181.47 ha), and low severity – 52.5 % (332.70 ha). The fire had the most significant impact on coniferous forests. Areas with a higher tree density covered by coniferous vegetation showed correspondingly higher burn severity. Deciduous and mixed vegetation, as well as agricultural lands located in the

Table 4. Thresholds for some indices.

from – to	dMIRBI	dNBR2	dBAIS2	dNBR
Unburn area	-0.755 $\div$ 0.349	-0.045 $\div$ 0.035	-0.360 $\div$ 0.225	-0.046 $\div$ 0.107
Low severity	0.350 $\div$ 0.944	0.036 $\div$ 0.092	-0.361 $\div$ -0.701	0.108 $\div$ 0.256
Medium severity	0.945 $\div$ 1.667	0.093 $\div$ 0.166	-0.702 $\div$ -1.081	0.257 $\div$ 0.441
High severity	1.668 $\div$ 2.857	0.167 $\div$ 0.268	-1.082 $\div$ -2.138	0.442 $\div$ 0.726

southern part of the study area, were affected to a lesser extent, showing low to moderate burn severity.

Figure 3 illustrates the map of the study area by burn severity derived using dMIRBI.

The map of the study area by burn severity derived using dMIRBI including a 'low severity' category reveals additional affected areas that are not reported on the official website of EFA. However, in relation to the total burned area in the EFFIS database, such a category should be present. This confirms that the use of spectral indices (such as dMIRBI) provides a more comprehensive and objective picture of

the fire's impact on the territory.

Comparison with existing source data

To compare the results obtained using spectral indices and to determine the areas affected by the fire, data from the European Forest Fire Information System (EFFIS) were used (Copernicus Emergency Management Service 2025). Table 5 presents the burned areas estimated using the selected spectral indices, compared to the area determined by EFFIS, along with the percentage difference between them.

Figure 4 illustrates the boundaries of

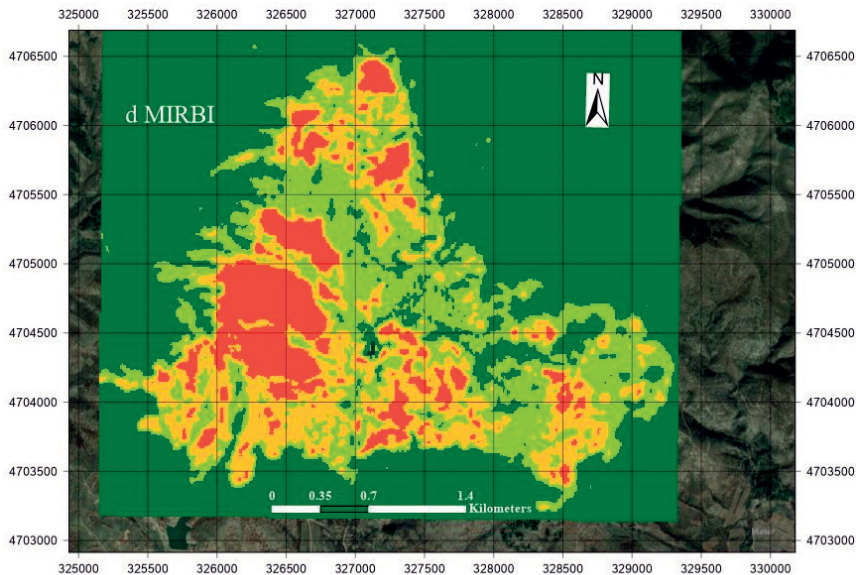


Fig. 3. Map of the study area by burn severity.

Table 5. Comparison of the burnt area estimates derived from the spectral indices with the highest separability values and the reference data from EFFIS.

Index	Area, ha	Area difference, ha	Area difference, %
Reference value (EFFIS)	634	-	-
dMIRBI	633.61	0.39	0.06
dNBR2	625.04	8.96	1.41
dNBR	622.27	11.73	1.86
dBAS2	620.04	13.96	2.20

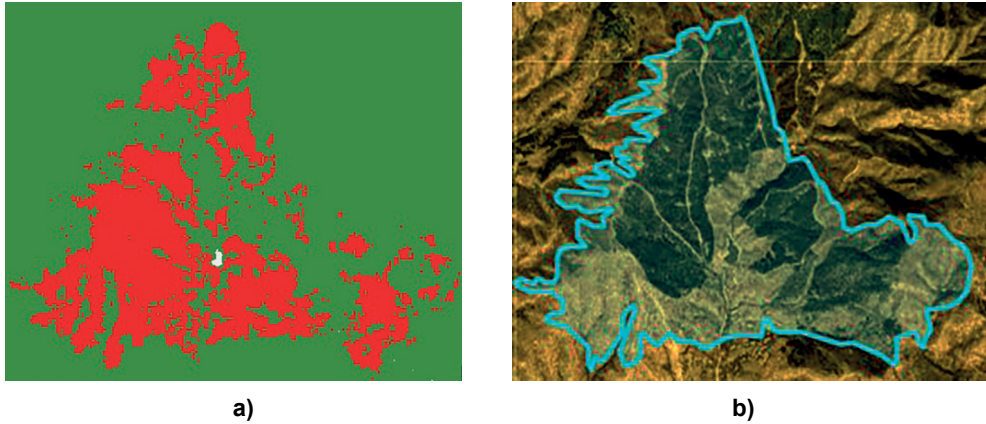


Fig. 4. Burnt area obtained from: a) dMIRBI and b) EFFIS.

the burnt areas derived using dMIRBI and those presented on the EFFIS website.

Based on the comparison of the burned areas, it can be stated that the differences between the indices with the highest separability values are minimal. Nevertheless, it should be noted that additional studies are necessary to confirm the reliability of the obtained results.

Forest stands often fall within the protected areas of Bulgaria. Wildfires in these regions can lead to the loss of priority habitats and disrupt the ecological processes taking place within them. Accurate localisation and measurement of the burned areas are essential for an objective assessment of the impact on biodiversity and for the development of effective strategies for the restoration of plant and animal life. For this reason, an analysis was conducted using data on the boundaries of protected areas provided by EFA. The analysis established that the studied site does not fall within any protected territory.

Conclusion

The study presents the results of applying various spectral indices, like NBR, NBR2,

BAI, BAIS2, MIRBI, RBR, and NDVI, to delineate burned areas' boundaries and determine burn severity. Based on a statistical evaluation using the separability index and on a comparison of the fire-affected areas, it was found that the BAIS2, MIRBI, NBR2, and NBR indices demonstrated the highest separability. Despite their close values, the MIRBI spectral index had the highest score in the present study (Table 4). This is consistent with other studies (Pacheco et al. 2023), which show that the reflectance values in the SWIR1 and SWIR2 bands represented in dMIRBI offer greater potential for discriminating and detecting changes in fire severity. This is also confirmed by the fact that the separability index values of NBR2 are very close to those of MIRBI for the study area.

Therefore, for detecting burned areas, we recommend the use of indices based on the two SWIR bands (Sentinel-2 bands – B11 and B12).

The results show that spectral indices are a valuable tool for locating and assessing burned areas in a mid-mountain region characterised by a temperate climate, forest vegetation, pastures, and meadows. These findings are supported by previous studies, which indicate that

the effectiveness and performance of spectral indices in distinguishing between burned and unburned areas strongly depend on the types of vegetation, as well as on the temporal intervals of the imagery and spectral ranges used.

The proposed methodology has the potential to be applied in other areas with analogous characteristics, thereby enhancing the reliability and generalisability of the obtained results. To reinforce the results of the present study related to the identification and mapping of burned areas and the adverse effects of those on vegetation, further research could be conducted based on these indices in other regions, thereby expanding the study area.

In the future, further in-depth studies could also be carried out with a focus on the analysis of fire severity across different vegetation types, as well as monitoring forest recovery after fire events.

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Structural fibre components of the cell walls of forage legume grasses grown in mountain areas

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Thematic field: Agriculture in mountainous and semi-mountainous regions

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Abstract

In the period 2020–2022 in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture – Troyan a research experiment has been conducted with the following species of perennial leguminous meadow grasses: bird's-foot-trefoil (*Lotus corniculatus* L.), hybrid clover (*Trifolium hybridum* L.), alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.). The experiment aimed to monitor the composition of the structural fibre components of the forage cell walls average two-year period. The dry matter digestibility and energy nutritional value was determined. The legume crops studied were found to have different compositions of forage cell wall fractions. The forage mass of red clover showed the lowest content of neutral and acid detergent fibre (30.87 % and 21.49 %), and acid detergent lignin (8.11 %) and the highest dry matter digestibility (73.11 %). The alfalfa forage had the highest hemicellulose values (11.17 %) and that of hybrid clover had the maximum cellulose values (24.06 %). Good energy nutritive value was recorded by the forage of bird's-foot-trefoil and white clover.

Key words: cell wall fibre components, digestibility, legume grasses.

Introduction

Legume meadow grass species are suitable components for the establishment of grass mixtures (Bozhanska et al. 2016, Kirilov 2016, Arlauskienė et al. 2021) due to their high productivity, ability to fix nitrogen from air and their nutritional value,

which defines them as good forage for farm animals (Churkova et al. 2016). They are less often grown alone because in combination with perennial grasses they have higher productivity and higher forage quality, lower nitrogen requirements, higher resistance to diseases and pests, and better seasonal distribution of the for-

age obtained (Annicchiarico 2008). The inclusion of legumes in the composition of grasses increases the quality characteristics of forage and maintains a sustainable balance between the perennial and legume components (Naydenova et al. 2010). For the mountain conditions of Bulgaria, suitable legumes for obtaining forage with high nutritional value are red clover (Naydenova and Bozhanska 2014, Britaňák et al. 2018), white clover (Mihovski and Sabeva 2011) and bird's-foot-trefoil (Churkova and Churkova 2022). Alfalfa is a source of plant protein that determines the nutritional value of forage under changing climatic conditions (Marinova 2020).

The cell wall component contents of legume forage grasses are important indicators of dry matter uptake and utilization depending on the grass species. They determine forage quality due to being a source of energy for ruminants when they break down (Ivanova et al. 2019). Detergent analysis based on fibre composition and digestibility is the basis for determining forage digestion (Marković et al. 2023).

Acid detergent fibre (ADF) determines feed digestibility and neutral detergent fibre (NDF) determines feed utilization (Ertekin et al. 2022). The amount of neutrally detergent fibre in forage of self-seeded cropped bird's-foot-trefoil was $418.9 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$ and was lower compared to growing it with perennial grasses (Churkova and Churkova 2020). In a study of self-seeded grassland from legume crops, Petkova et al. (2022) found that white clover had the highest dry matter digestibility ($722.63 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$), red clover had the highest neutral-detergent fibre content ($418.03 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$), and bird's-foot-trefoil biomass had the highest concentration of acid-detergent fibre ($291.98 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$), acid-detergent lignin ($70.72 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$), and cellulose

($221.27 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$).

The study aimed to determine the fibre composition of cell wall components and the energy value of forage from perennial legume grasses grown under mountain conditions.

Material and Methods

The research experiment was carried out in 2020 in the experimental field of Research Institute of Mountain Stockbreeding and Agriculture, Troyan. The area is located at the northern foot of the Central Stara Planina Mountains (Bulgaria) at an altitude of 384 m a.s.l. The experiment includes the following species of meadow legume crops: bird's-foot-trefoil (*Lotus corniculatus* L.), hybrid clover (*Trifolium hybridum* L.), alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.) on light gray pseudopodzolic soil.

The experiment was laid out using the block method in four replications with a harvest plot size of 5 m^2 . A generally accepted technology for the cultivation of meadow legume crops for forage was applied (Churkova and Churkova 2024). The sowing rate for grass species was as follows: for bird's-foot-trefoil, white and hybrid clover – $12 \text{ kg}\cdot\text{ha}^{-1}$, for alfalfa and red clover – $25 \text{ kg}\cdot\text{ha}^{-1}$. The sowing of the grassland was carried out manually, scattered at different seeding rates according to the grass species.

Samples for chemical analysis were determined for two consecutive years (2021 and 2022) and were averaged over the period.

The harvesting of the leguminous meadow grasses (*Lotus corniculatus*, *Medicago sativa*, *Trifolium hybridum*, *T. pratense*, *T. repens*) was carried out in

June at the beginning of flowering.

Samples were taken immediately before harvesting in the budding-early flowering stage. Dried samples were ground with a grinder through a 1 mm mesh sieve before analysis. The structural carbohydrates of the cell walls were determined in each sample, the nutritive value and *in vitro* enzymatic digestibility of the dry matter were calculated.

The fibre composition of the cell walls was determined in % and included:

- NDF;
- ADF;
- Acid-detergent lignin (ADL) – determined by the detergent analysis of Van Soest et al. (1991);
- Degree of lignification (expressed as percentage of ADL/NDF);
- Hemicellulose (calculated by NDF–ADF);
- Cellulose = ADF–ADL.

Nutritional value of the forage was estimated using the Bulgarian system as feed units for milk (FUM) and feed units for growth (FUG) and calculated based on equations (1) and (2) according to the experimental values of CP, CF, CF and NFE, recalculated using the digestibility factors proposed by Todorov et al. (2010).

$$GE = 0.0242 \cdot CP + 0.0366 \cdot CF + 0.0209 \cdot CF + 0.017 \cdot NFE - 0.0007 \cdot Zx, \quad (1)$$

where: GE is gross energy, MJ·kg⁻¹ DM; CP is crude protein, %; CF is crude fibre, %; NFE is nitrogen free extractable substances, %; Zx is sugars, %.

$$EE = 0.0152 \cdot DP + 0.0342 \cdot D + 0.0128 \cdot DF + 0.0159 \cdot DNFE - 0.0007 \cdot Zx, \quad (2)$$

where: EE is exchangeable energy, MJ·kg⁻¹ DM; DP is digestible protein, %; D is digestibility, %; DF is digestible fibre, %; DNFE is digestible nitrogen free extractable substances, %; Zx is sugars, %.

FUM and FUG were calculated using

equations (3) and (4), respectively.

$$FUM = EE \cdot (0.075 + 0.039 \cdot q), \quad (3)$$

$$FUG = EE \cdot (0.04 + 0.1 \cdot q), \quad (4)$$

where: *q* is exchange rate.

In vitro enzyme digestibility of dry matter (%) was determined according to Aufrere (1982) by the two-phase pepsin-cellulose method. In the first phase, a pre-treatment with pepsin-hydrochloric acid was carried out to digest the protein, and in the second phase, the residue of the feed was treated in an acid condition with the enzyme cellulose to digest the cellulose.

Statistical treatment of data is represented by mean (X), standard deviation (SD); minimum (min) and maximum (max) limits (Lidanski 1988). The degree of variation of the parameters of individual indicators was determined by the coefficient of variation (CV) according to Mamaev's scheme: up to 7 % – very low; above 7.0 to 12.0 % – low; above 12.0 to 20.0 % – moderate; above 20.0 to 40.0 % – high; above 40.0 % – very high. The CV measures the variability relative to the mean and is found by dividing the SD by the mean. For this purpose, the software products Analysis Toolpak for Microsoft Excel 2010 and Statgraphics Plus v.2.1 were used.

Results and Discussion

To estimate the forage quality of different meadow legume crops in Table 1 are presented data for neutral and acid detergent fibre, ADL, hemicellulose and cellulose.

Cell wall components of legume crops are closely related to variety, stem anatomy (Engels and Jung 1998), and stage of plant development (Doblin et al. 2010). NDF is a key indicator for predicting the

Table 1. Structural fibre components of cell walls of forage from meadow legume crops averaged over the period 2021–2022.

Variants	NDF, %	ADF, %	ADL, %	Hemicellulose, %	Cellulose, %	Degree of lignification (coefficient)
<i>Lotus corniculatus</i> L.	45.18	34.90	13.17	10.28	21.74	28.79
<i>Trifolium hibridum</i> L.	43.34	33.07	9.02	10.26	24.06	20.70
<i>Medicago sativa</i> L.	42.21	31.04	11.25	11.17	19.80	26.06
<i>Trifolium pratense</i> L.	30.87	21.49	8.11	9.38	13.38	27.20
<i>Trifolium repens</i> L.	37.08	27.80	8.20	9.28	19.60	22.09
X	39.73	29.66	9.95	10.07	19.71	24.97
SD	5.80	5.27	2.20	0.77	3.97	3.44
CV	14.59	17.78	22.10	7.67	20.16	13.78
Min	30.87	21.49	8.11	9.28	13.38	20.70
Max	45.18	34.90	13.17	11.17	24.06	28.79

free uptake of forage by animals, while ADF influences forage degradability and digestibility. Red clover had the lowest content of NDF (30.87 %), ADF (21.49 %) and ADL (8.11 %).

Second place in terms of indicators defining a balanced quality composition of the forage was occupied by white clover, with NDF 37.08 %, ADF 27.80 % and ADL 8.20 %, respectively. More significant changes were observed in NDF, ADF and ADL contents of bird's-foot-trefoil forage mass compared to those of other legumes. The mean excess of NDF was 14.31 %, of ADL – 13.41 %, and of ADF – 5.06 % compared with the values of red clover forage. Hybrid clover had elevated values for these three parameters. NDF exceeded those of red clover by 12.47 % and white clover by 6.26 %. The same trend was observed for ADF and ADL content. Lignin plays an important role in structural integrity of the cell wall, stem strength, water transport, mechanical support and protection against plant pathogens (Wang et al. 2013, You et al. 2013), but has an abrasive effect on digestion by degrading plant cell walls (Lamb et al. 2007). This requires a search for crop species with reduced lig-

nin content with appropriate composition leading to increased lignin degradation and increased energy efficiency of the forage (Jung and Engels 2002).

Therefore, the forage mass of red clover has the most suitable quality composition in terms of lignin due to its reduced content of 8.11 %. Bird's-foot-trefoil had the highest levels of NDF, ADF and ADL accordingly: 45.18 %, 34.90 %, 13.17 %. Pavlov and Naydenova (2000) found that the content of the three components increased with increasing age of the bird's-foot-trefoil. Alfalfa had values of these parameters higher than those of white and red clover, and lower than those of bird's-foot-trefoil. According to the data obtained on CV, neutral (CV = 14.59 %) and ADF (CV = 17.78 %), have a medium degree of variability, and ADL has a high degree of variability (CV = 22.10 %). These results indicate a significant difference among the species of legume crops in relation to ADL and less in relation to neutral and acid detergent fibres.

Forage lignification correlates negatively with its digestibility. Feed uptake depends not only on the components of the cell walls, but also on their volume

and degradability. The degree of lignification had the highest values for the bird's-foot-trefoil forage (28.79) and the lowest for the hybrid clover forage (20.70). Vasileva and Naydenova (2017) also obtained high values for this indicator of fodder from self-seeded bird's-foot-trefoil. The degree of lignification of red clover slightly exceeded that of alfalfa, by 1.14. White clover forage (22.09) had higher values for degree of lignification than hybrid clover. Hemicellulose had similar values in the forage mass of red (9.38 %) and white (9.28 %) clovers, as well as bird's-foot-trefoil (10.28 %) and hybrid clovers (10.26 %). The highest value was recorded for the fodder mass of alfalfa (11.17 %) with an average value of $X = 10.07$ %.

The variation of hemicellulose in the forage of the studied meadow legume grasses according to the CV had a very low degree of variability ($CV = 7.67$ %). The cellulose polysaccharide content varied from 13.38 % to 24.06 %, with a maximum value in hybrid clover and a minimum in red clover. The average value

of cellulose content was 19.71 %, and according to the CV, the degree of variability of this indicator was high ($CV = 20.16$ %). Of the meadow legume grasses studied, red clover forage had the most favourable cellulose content regarding forage quality.

Feed intake is considered to be physiologically regulated if the digestibility of organic substance is lower than 67 % (van Vuuren 1994), and higher digestibility is an indicator of high feed quality. In our study, high values for the digestibility (Fig. 1) of the dry matter of the feed of red clover (73.11 %) and white clover (68.13 %) were obtained. The lowest digestibility of dry matter is the feed mass of bird's-foot-trefoil (62.54 %), which is in line with the high levels of neutral and acid-detergent fibres and ADL, which corresponded to an established relationship between the digestibility of dry matter and the fibrous components of cell walls (Naydenova and Pavlov 2005, Glindemann et al. 2009).

This was confirmed by the highest digestibility of red clover feed, which had the lowest values of NDF, ADF and ADL,

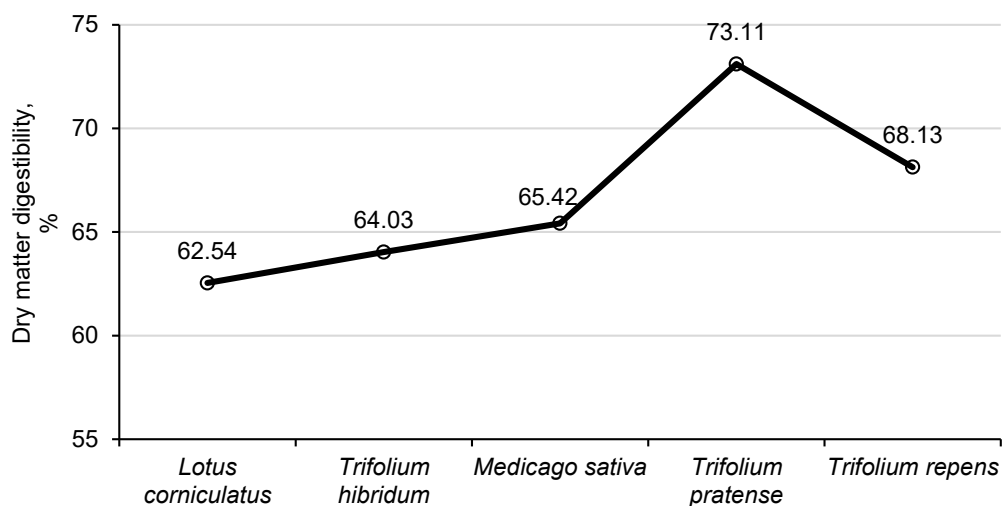


Fig. 1. Dry matter digestibility of meadow legume grass forage.

Note: Dry matter digestibility determined according to the method of Aufrere (1982).

and counter the feed mass of bird's-foot-trefoil, which had the highest content of these indicators, had the lowest digestibility. A trend was observed that proves that with an increase in CF, NDF and ADF, the enzymatic digestibility of the dry matter decreases, and with an increase in CP, the digestibility increases (Stoycheva et al. 2016). Hybrid clover (64.03 %) and alfalfa (65.42 %) have almost similar values of digestibility of the dry matter of the feed. The relative and absolute proportion of digestible cell content decreases rapidly during the growth of grass species, while the proportion of ADF, CF and hemicellulose increases slowly and then remains at the same level (Carlier et al. 2011). The absolute proportion of digestible cell walls in the total dry matter content reaches a maximum in the middle of the development phase, then decreases.

Energy nutritional value determines the balance between the quantity and quality of the forage (Vasileva and Naydenova 2017). The difference between the gross and exchangeable energy of forage (Table 2) from legume grasses ranged from 17.39 to 17.90 MJ·kg⁻¹ DM, with the highest value for forage from bird's-foot-trefoil. The same trend was observed for exchange energy.

FUM in bird's-foot-trefoil and white clover forages were similar (0.70 pieces) and alfalfa forage recorded the lowest values

(0.62 pieces). FUG of forage from white clover were highest (0.64 pieces), followed by the forage mass of bird's-foot-trefoil (0.63 pieces) and lowest for alfalfa (0.54 pieces), with the trend being identical to that of feed units for milk. Vateva and Stoeva (2007) obtained values of FUM from a self-seeded bird's-foot-trefoil – 0.698 pieces, and of FUG – 0.590 pieces, which are close to those obtained by us. According to literature sources (Carlier et al. 2011), the FUM of alfalfa varies from 0.6 to 0.9 pieces in 1 kg of dry matter, and in the scientific experiment the reported value is 0.62 pieces, the difference being due to the vegetation phase. The most favourable balance between quality and yield is obtained in the early flowering phase when the stands of the studied legume grasses are harvested.

Conclusions

The meadow legume grasses from bird's-foot-trefoil, hybrid clover, alfalfa, red and white clover showed different composition of forage cell wall fractions. The forage mass of red clover registered the lowest content of neutral and acid detergent fibres, and acid detergent lignin. The high values of the same parameters of the forage of bird's-foot-trefoil make it less suitable for cultivation compared to the rest

Table 2. Energy nutritional value of meadow legume grass feed.

Legume grasses	GE, MJ·kg ⁻¹ DM	EE, MJ·kg ⁻¹ DM	FUM, numbers	FUG, numbers
<i>Lotus corniculatus</i> L.	17.90	7.66	0.70	0.63
<i>Trifolium hibridum</i> L.	17.39	7.48	0.69	0.62
<i>Medicago sativa</i> L.	17.49	6.83	0.62	0.54
<i>Trifolium pratense</i> L.	17.84	7.24	0.66	0.58
<i>Trifolium repens</i> L.	17.51	7.64	0.70	0.64

Note: GE is gross energy, EE is exchangeable energy, FUM is feed units for milk, FUG is feed units for growth.

tested legume grasses in terms of forage quality. Alfalfa forage had the highest hemicellulose values and that of hybrid clover had the maximum cellulose values.

Low values of indicators of neutral and acid detergent fibre and acid detergent lignin determine the high dry matter digestibility of red clover forage.

The high crude protein content of bird's-foot-trefoil and white clover fodder predetermines the high nutritional value of their fodder.

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Dietary composition of Common Wood pigeon (*Columba palumbus*) in Bulgaria

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Thematic field: Wildlife conservation and management

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Abstract

Common Wood pigeon (*Columba palumbus* L., 1758) is a widespread species in Europe including Bulgaria, with increasing population trend. Understanding its diet is essential for assessing its ecological role and predicting responses to habitat changes. This study examines the feeding spectrum in autumn and winter of the species in Bulgaria based on crop content analysis of 54 specimens collected from 11 hunting areas between 2022 and 2024. A total of 21 dietary components were identified, including 17 plant and 4 animal taxa. The primary food resources were sunflower seeds, maize, and acorns, with seasonal variations in diet composition. The study confirms the opportunistic feeding behaviour of the Wood pigeon, influenced by food availability. While cereals and acorns remain staple components, molluscs and insects played a minor role. These findings contribute to a better understanding of the species' feeding habits in Bulgaria and provide a basis for future ecological and conservation research.

Key words: acorns, crop damage, human-wildlife conflict, seeds.

Introduction

Common Wood pigeon (*Columba palumbus* L., 1758) is naturally distributed throughout Europe, parts of Western Asia and Northern Europe, including Bulgaria (BirdLife International 2018). It falls into the category of Least Concern with a growing population according to IUCN criteria (BirdLife International 2018). The European population is estimated to be between 20.5 and 29 million pairs and accounts for 80 % of the global population of the species (BirdLife International 2018). The expansion of agriculture in the 19th century contributed to the species' increasing number (Marchant et al. 1990). This also led to an increase in suburban and urban areas (Slater 1995, Sakhvon and Kövér 2020). Today, the Wood pigeon is a common species in many European cities with high density of? (Bea et al. 2011, Zbyryt 2014). Urban populations are increasing across Europe, with observed differences in nest-site selection (Gil et al. 2024). The species is among the most frequently recorded birds in urban areas of Mediterranean cities during the summer (Luna and Blanch 2024). During this process of synanthropization, habitat use changes compared to that in rural areas (Frey et al. 2015). The growth of urban Wood pigeon population has been associated with the absence of nest predators, adaptation to human-related food resources, and access to open agricultural land within the species' economic range (Frey et al. 2015, Tomiałojć 2021). In the period 2005–2010, the species had an increasing population in Bulgaria (Spasov et al. 2017). According to a twenty-year study of widespread bird's species, it is also classified as a species with an increasing population trend (Hristov et al. 2023). Wood pigeon is considered a widespread species,

found in forest, shrub and anthropogenic habitats (Baptista et al. 1997). Since the mid-1970s, the increase of its population has been associated with the introduction of oilseed rape (*Brassica napus* L.) as a widespread field crop. At the end of the last century, the species has already been defined as an agricultural pest in some parts of its range (Inglis et al. 1994, 1997).

Determining the diet of a species is important for many population aspects and its management. Food resources directly affect the distribution and breeding potential (Mengesha and Bekele 2008, Ruffino et al. 2014, Sam et al. 2017). Reduced food availability during winter can directly affect the survival of adult birds and subsequently affect breeding potential (Danner 2012, Plummer et al. 2013). Reduced food availability during the breeding season can directly affect breeding success (Brinkhof and Cavé 1997). The leading food components in the Common Wood pigeon diet are seeds, fruits and leaves (Ó hUallachain and Dunne 2013, Gutiérrez-Galán et al. 2017). The season, the study area and the type of crops grown in the agricultural lands have a great influence on its food spectrum (Ó hUallachain and Dunne 2013). During the spring – summer season, the main share of the species' food is occupied by wheat seeds, supplemented with sunflower seeds in the autumn. In winter, the main share is occupied by acorns (Purroy et al. 1984, Jimenez et al. 1994, Kaouchi et al. 2021). In Bulgaria, the food spectrum has been poorly studied, and from 28 stomachs studied, mainly Hawthorn (*Crataegus monogyna* Jacq.), Dog rose (*Rosa canina* L.), Sloe (*Prunus spinosa* L.), and oak and beech acorns, weeds (Simeonov et al. 1990) were found. In the last two decades, the food has not studied in Bulgaria.

The aim of this study is to present

novel insights into the autumn-winter diet of the Common Wood pigeon (*Columba palumbus* L.) in Bulgaria, in order to enhance our understanding of its ecological requirements and adaptive strategies in response to habitat alternations.

Materials and Methods

Between September 2022 and December 2024, we analysed the crops of 54 Wood pigeons collected from eleven hunting areas (Fig. 1). The collected samples for analysis were obtained from hunting grounds located within the species' natural range in Bulgaria, outside of human settlements. The habitats in the sampling areas primarily consist of lowland deciduous forests, adjacent to various types

of agricultural land, which provide a diverse range of food resources. The relief is predominantly flat, primarily occupied by arable land. The main cultivated crops include maize, wheat, sunflower, and barley. Forest habitats are represented by small lowland broadleaf woodlands and scattered patches of conifer plantations, mainly Scots pine (*Pinus sylvestris* L.) and black locust (*Robinia pseudoacacia* L.). The lowland broadleaf forests are predominantly composed of Turkey oak (*Quercus cerris* L.) and Oriental hornbeam (*Carpinus orientalis* Mill.), occasionally interspersed with Manna ash (*Fraxinus ornus* L.). Another type of broadleaf forest typical of the region consists of mixed stands of Turkey oak and *Quercus virgiliana* Ten. Additionally, mixed forests composed of Silver linden (*Tilia tomentosa*

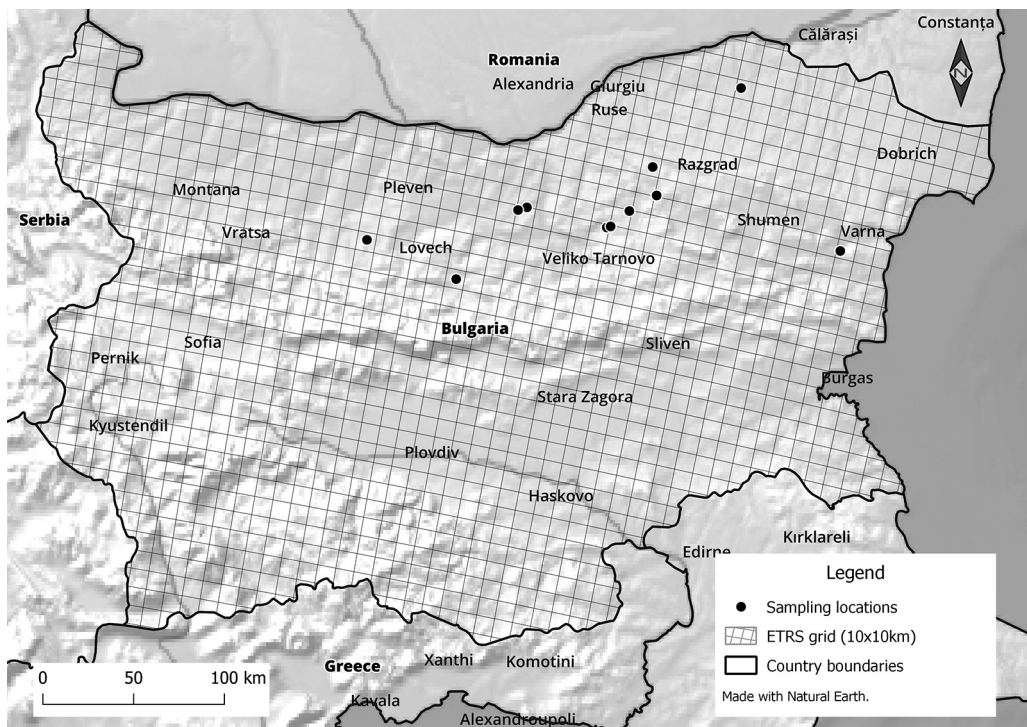


Fig. 1. Wood pigeon sampling areas during the period 2022–2024.

Moench) and European hornbeam (*Carpinus betulus* L.) are represented, as well as silver linden mixed with Turkey oak, occasionally accompanied by Hungarian oak (*Quercus dalechampii* Ten.) and Field maple (*Acer campestre* L.). A small part of the studied quadrants is occupied by pastures, which are characterised as xerothermic grassland formations, dominated by plant communities *Dichanthietum ischaemi* Knapp ex Oberd. 1954, *Poaetum bulbosae* and *Poaetum concinnae* Soó 1964, *Chrysopogoneta grylli* Horvat 1951, and ephemeral communities (*Ephemeret*) (Bondev 1991).

All specimens were obtained during regular hunting trips in September 2022, September-December 2023, and January 2024. The contents of each crop were extracted, sorted by species, and air-dried for two weeks. Each fraction was then weighed using an electronic scale with an accuracy of 0.001 g (KERN PLJ-N (KERN & Sohn GmbH)). The dietary components were categorised into plant, animal, and inorganic matter. Plant material was identified using a LEICA EZ4 W trinocular stereoscope and a reference collection. Additionally, the identification keys of Franke et al. (1979), Chepik (1981), and Artjuschenko and Teodorov (1986) were used. For acorns, the identifier, according to the

key by Schwartz (1937), was also used.

The snail species were identified by D. Georgiev using the author's reference shell collection. The significance of each dietary component was calculated using the formula (1) (Mihaylov 1995).

$$\text{Significance} = \frac{\% \text{ occurrence} \cdot \% \text{ weight}}{\sum (\% \text{ occurrence} \cdot \% \text{ weight})}, \% \quad (1)$$

where: % occurrence – the percentage of crops containing the given dietary component; % weight – the mean weight of the given component relative to the average total crop content.

The percentage contribution of food items was calculated so that the data can be compared with these of previous studies (Murton et al. 1964, Ó hUallachain and Dunne 2013, Gutiérrez-Galán et al. 2017, Luna et al. 2024).

Results

A total of 21 dietary components were identified, including 17 of plant and 4 of animal origin. Sunflower seeds had the highest occurrence rate (47.7 %), while maize seeds had the highest significance percentage (31.3 %), followed by acorns from various oak species (29.1 %) (tables 1 and 2).

Table 1. Taxonomic composition of dietary components identified in the crop contents of Wood pigeons.

No	Food spectrum	Family	Order
Tree species			
1	Silver birch (<i>Betula pendula</i> Roth.)	Betulaceae	Fagales
2	Hungarian oak (<i>Quercus frainetto</i> Ten.)	Fagaceae	Fagales
3	Downy oak (<i>Quercus pubescens</i> Willd.)	Fabaceae	Fabales
4	Black locust (<i>Robinia pseudoacacia</i> L.)	Fabaceae	Fabales
5	Undetermined species Fabaceae	Fabaceae	Fabales
6	Willows (<i>Salix</i> sp.)	Salicaceae	Malpighiales

No	Food spectrum	Family	Order
Shrub species			
1	Roses (<i>Rosa</i> sp.)	Rosaceae	Rosales
2	Common ivy (<i>Hedera helix</i> L.)	Araliaceae	Apiales
3	Undetermined fruit	N/A	N/A
Grass species			
1	Centaury (<i>Centaurea</i> sp.)	Asteraceae	Asterales
2	Barnyard grass (<i>Echinochloa crus-galli</i> (L.) P.Beauv.)	Poaceae	Poales
3	Common knotgrass (<i>Polygonum aviculare</i> L.)	Polygonaceae	Caryophyllales
4	Hairy vetch (<i>Vicia villosa</i> Roth)	Fabaceae	Fabales
5	Alfalfa (<i>Medicago sativa</i> L.)	Fabaceae	Fabales
6	<i>Polygonum</i> sp.	Polygonaceae	Caryophyllales
7	Undetermined cereal grasses	Poaceae	Poales
8	Undetermined grass	N/A	N/A
Agricultural crops			
1	Sunflower (<i>Helianthus annuus</i> L.)	Asteraceae	Asterales
2	Common Wheat (<i>Triticum aestivum</i> L.)	Poaceae	Poales
3	Maize (<i>Zea mays</i> L.)	Poaceae	Poales
4	Common oat (<i>Avena sativa</i> L.)	Poaceae	Poales
5	Common barley (<i>Hordeum vulgare</i> L.)	Poaceae	Poales
Snails			
1	<i>Morlina glabra</i> Rossmassler, 1835	Oxychilidae	Stylimmatiohora
2	<i>Pomatias rivularis</i> Eichwald, 1829	Pomatiidae	Littorinimorpha
3	Species Hygromiidae 1	Hygromiidae	N/A
4	Species Hygromiidae 2	Hygromiidae	N/A
5	Undetermined species Mollusca	N/A	N/A
Insects			
1	Unidentified insects	N/A	N/A
Inorganic component			
1	Gastroliths		
2	Soil		
3	Cable insulation		

Note: Unidentified species Hygromiidae 1 and 2 are species in this family but they were not identified to species taxon. The last 3 rows of the table contain empty columns for family and order because inorganic parts are not classified this way.

Table 2. Frequency, mean weight, and relative nutritional importance of food items identified in the crop contents of Wood pigeons.

No	Food spectrum	Occurrence, %	Mean weight, g	Significance, %
1	Black locust (<i>Robinia pseudoacacia</i> L.) – seeds	6.8	0.048	0.570
2	Hungarian oak (<i>Quercus frainetto</i> Ten.) – acorns	9.1	12.021	18.947

No	Food spectrum	Occurrence, %	Mean weight, g	Significance, %
3	Downy oak (<i>Quercus pubescens</i> Willd.) – acorns	6.8	8.558	10.117
4	Other oaks (<i>Quercus</i> sp.) – acorns	2.3	0.226	0.089
5	Willows (<i>Salix</i> sp.) – seeds	2.3	0.003	0.001
6	Ivy (<i>Hedera helix</i> L.) – fruit	6.8	1.265	1.495
7	Ivy (<i>Hedera helix</i> L.) – leaf	2.3	0.010	0.004
8	<i>Rosa</i> sp. – fruit	2.3	0.610	0.240
9	Centaury (<i>Centaurea</i> sp.) – leaf	2.3	0.000	0.000
10	Barnyard grass (<i>Echinochloa crus-galli</i> (L.) P. Beauv.) – seeds	2.3	0.729	0.287
11	Knotgrass (<i>Polygonum aviculare</i> L.) – seeds	2.3	0.180	0.071
12	Hairy vetch (<i>Vicia villosa</i> Roth) – seeds	2.3	0.208	0.082
13	Cereal grasses – leaf	20.5	0.037	0.131
14	Undetermined grass – leaf	31.8	0.041	0.226
15	Sunflower (<i>Helianthus annuus</i> L.) – seed husks	4.5	0.014	0.011
16	Sunflower (<i>Helianthus annuus</i> L.) – leaf	2.3	0.004	0.002
17	Sunflower (<i>Helianthus annuus</i> L.) – seeds	47.7	3.258	26.960
18	Common wheat (<i>Triticum aestivum</i> L.) – leaf	2.3	0.030	0.012
19	Common oat (<i>Avena sativa</i> L.) – seed husks	2.3	0.000	0.000
20	Common barley (<i>Hordeum vulgare</i> L.) – seed husks	2.3	0.000	0.000
21	<i>Polygonum</i> sp. – seeds	2.3	0.009	0.004
22	Alfalfa (<i>Medicago sativa</i> L.) – leaf	20.5	0.051	0.181
23	Common wheat (<i>Triticum aestivum</i> L.) – seeds	20.5	2.598	9.214
24	Maize (<i>Zea mays</i> L.) – seeds	22.7	7.952	31.335
25	<i>Morlina glabra</i> Rossmassler, 1835	2.3	0.012	0.005
26	<i>Pomatias rivularis</i> Eichwaid, 1829	2.3	0.021	0.008
27	unidentified Fabaceae – leaf	2.3	0.021	0.008
28	Unidentified snail 1	2.3	0.026	0.010
29	Unidentified snail 2	2.3	0.005	0.002
30	Unidentified snail 3	2.3	0.025	0.010
31	Unidentified snail 4	2.3	0.000	0.000
32	Unidentified snail 5	2.3	0.013	0.005
33	Unidentified insects 1	2.3	0.000	0.000
34	Unidentified insects 2	2.3	0.000	0.000
Inorganic components				
35	Gastroliths	15.9	0.114	0.314
36	Soil	9.1	0.107	0.169
37	Cable insulation	2.3	0.000	0.000
Undetermined				
38	Unidentified fruit 1	2.3	0.012	0.005
Mean				2.632

Note: The unidentified snails and insects indicated by numbers 1, 2, etc., are of different species, but due to remnants of shell parts, they could not be identified.

The animal component of the Wood pigeon's diet included several snails (Molluscs) species, of which only two were identified: *Morlina glabra* and *Pomatias rivularis*. However, their contribution to the overall diet was minimal.

Discussion

This study identified 16 food species in the diet of the Wood pigeon during the autumn-winter period. An additional 11 food components remained unidentified (Table 1). The results indicate that the primary food sources are seeds from various agricultural crops and acorns from different oak species.

The diet of the Wood pigeon has been relatively well studied across most of its range, with plant species forming the bulk of its food intake. However, significant variations in species composition occur depending on the availability and accessibility of specific food sources (Glutz von Blotzheim et al. 1986). Recent studies have shown that in some parts of Ireland, beech acorns are the dominant food source, whereas in southern Spain, oak acorns are the most commonly consumed (Ó hUallachain and Dunne 2013, Gutiérrez-Galán et al. 2017). Our findings support previous research highlighting the importance of acorns in the Wood pigeon diet (Table 2). In this study, acorns were among the most significant food sources, playing a major role in the winter diet (Purroy et al. 1984, Jimenez et al. 1994).

In Algeria, studies have found that seeds from the genera *Oxalis*, *Hordeum*, and *Triticum*, among others, contribute significantly to the diet (Guezzoul et al. 2004, Merabet et al. 2014). Further research in Eastern Algeria has identified a

variety of food sources, including Juniper berries (*Juniperus communis* L.), Cork oak (*Quercus suber* L.), Holm oak (*Quercus ilex* L.), Algerian oak (*Quercus canariensis* Willd.), Aleppo pine (*Pinus halepensis* Mill.), White willow (*Salix alba* L.), olive (*Olea* sp.), as well as ash seeds, weeds, cereals, oats, clover, wheat, and barley (Kaouachi et al. 2021).

Naturally, the Wood pigeon's diet varies across seasons, reflecting fluctuations in food availability and diversity (Glutz von Blotzheim et al. 1986, Ó hUallachain and Dunne 2013). However, cereals, acorns, and various tree fruits remain staple food sources throughout most of the year (Gutiérrez-Galán et al. 2017, Fazekas 2023). These findings are further confirmed by the present study (Table 2). Previous research in southern Europe has highlighted the importance of cereal seeds such as wheat, barley, and sunflower, particularly in late summer. Bioclimatic differences within the Wood pigeon's range also contribute to dietary variations across habitats. For instance, while sunflower seeds form a significant part of the species' diet in southern Spain (Jimenez et al. 1994), they are consumed less frequently in central Europe (Ó hUallachain and Dunne 2013). In the present study, sunflower seeds were notably prevalent in the diet (Table 2). However, it should be noted that part of the data collection period coincided with the sunflower harvest. This seasonal factor likely influenced the observed high consumption, as Wood pigeons tend to gather in areas with abundant food resources, supporting the idea that they opportunistically exploit widely available food sources.

Despite this, the results reinforce the importance of cereals, which remain a staple component of the Wood pigeon's diet, consistent with findings from other stud-

ies (Jimenez et al. 1994, Gutiérrez-Galán et al. 2017). Alfalfa leaves had an occurrence of 20.5 % in our study but showed low dietary significance (0.18 %) (Table 2). This low significance is likely attributed to the season in which the study was conducted. Similar studies have reported high consumption of species from the genera *Trifolium* and *Medicago*, with their contribution reaching 44.2 % in spring and an average of 12.7 %. Clovers (*Trifolium* sp.) can constitute up to 22 % of the diet in summer, with an occurrence rate of up to 61 % (Ó hUallachain and Dunne 2013, Gutiérrez-Galán et al. 2017). The lower values observed in our study are likely due to seasonal variation and the limited availability of these food sources during the autumn-winter period.

Weed seeds and various fruits were recorded at low occurrence rates and had a low significance percentage in this study. This aligns with previous research indicating that such food sources were as secondary resources when seeds and acorns are available, becoming more important only when primary food sources are scarce (Jimenez et al. 1994, Ó hUallachain and Dunne 2013, Perea and Gutiérrez-Galán 2015).

Animal-based food is poorly represented in the diet of the Wood pigeon across most of its range. According to our results, the invertebrate component of its diet includes two unidentified insect species and five snail species, three of which could not be identified at the species level (Table 1). Their occurrence and dietary significance were both low (Table 2).

Various insects have been recorded in the Wood pigeon's diet, sometimes in substantial quantities (Glutz von Blotzheim et al. 1986). However, the limited presence of insects in this study is likely due to the seasonal timing of sample collection.

Snails (Molluscs) were also found in small amounts, which align with previous studies suggesting their role in calcium balance. The consumption of snails during the summer may be linked to increased protein and calcium requirements during the breeding season (Glutz von Blotzheim et al. 1986, Graveland 1996, Graveland and Drent 1997, Ó hUallachain and Dunne 2013, Gutiérrez-Galán et al. 2017). Additionally, the presence of molluscs in the diet of juvenile birds may indicate a specific dietary preference at an early stage of development (Gutiérrez-Galán et al. 2017). Different snail species have been recorded in various parts of the Wood pigeon's range, including Decollate snail (*Rumina decollata* L.), *Ferussacia folliculum* (Schröter, 1784), *Microxeromagna armillata* (Lowe, 1852), *Cochlicella barbara* (Linnaeus, 1758), and *Candidula gigaxii* (Pfeiffer, 1847) (Gutiérrez-Galán et al. 2017). Our study expands this list by documenting *Morlina glabra* and *Pomatias rivularis* as new additions to the known diet of the Wood pigeon.

The results of this study are consistent with findings from other parts of the species' European range, reinforcing the observation that Wood pigeons tend to gather around abundant and easily accessible food sources. While some dietary variations were noted across different regions and seasons, these were primarily driven by fluctuations in food availability. Overall, our findings support the idea that the Wood pigeon has a broad and flexible dietary niche, similar to patterns observed elsewhere in its range.

Conclusions

The present study confirms that the diet of the Wood pigeon during the autumn

– winter period is primarily composed of plant-derived food items. Three components alone accounted for more than 85 % of the total dietary significance (Table 2), indicating their crucial role in fulfilling the energetic and nutritional needs of the species in this season. The strong preference for agricultural seeds, underscores the species' opportunistic foraging behaviour and its tendency to exploit abundant and accessible food sources (e.g., post-harvest fields). The relatively high occurrence of acorns (19.1 % combined significance from *Quercus* spp.) reaffirms their importance as a staple autumn-winter food resource, especially in forest-edge habitats. From a management perspective, two key recommendations emerge: Preservation of mixed landscapes combining farmland with native oak woodlands can help sustain populations by ensuring both crop and wild food resources throughout the year; Delaying deep tillage or plowing after harvest could prolong access to residual seeds for granivorous birds during late autumn and winter, reducing nutritional stress in critical periods.

Future studies should explore seasonal shifts in dietary composition with higher temporal resolution, especially in relation to agricultural practices and habitat structure, to better understand how land use influences trophic dynamics and bird distribution.

Conflict of Interest

The authors declare that there is no conflict of interest.

All materials were collected in accordance with the legislation of the Republic of Bulgaria.

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Effect of mulch type, seed size, and plant adaptive strategies on germination and species composition in newly established perennial wildflower meadows

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Abstract

Ecological information about plant communities can contribute to better landscape design. Grime's CSR model, which categorises plant species as competitors (C), stress-tolerators (S), and ruderals (R), is fundamental to understanding plant community assembly. Seed size is a crucial trait that influences germination success and species establishment, especially when seeds are sown *in situ*. In landscape architecture, mulches are commonly used for sowing because they help retain soil moisture and suppress weed growth. This study investigates two main aspects in combination with different mulching substrates: (1) how CSR (competitor–stress-tolerator–ruderal) strategies influence species composition, and (2) how seed size affects initial germination. It also examines which adaptive strategies are associated with species that become dominant under specific mulch conditions. An experimental study was conducted using two types of mulch – organic (compost) and mineral (sand) – and three different seed mixtures containing perennial herbaceous plants. Species cover was assessed in the third year after sowing. Additional data on seed weight, adaptive strategy, and maximum species height were retrieved from published databases. The statistical analyses were performed using generalised linear models. The findings highlight the role of adaptive strategy in determining species dominance. The type of mulch affects the dominant adaptive strategy. Dominant plants in sand mulch had a higher R-score. In compost, the C-score was the highest, whereas in plots without mulch, the S-score prevails. Seed size also played a significant role in germination across various substrates. These results have important implications for species selection in wildflower meadow design. Understanding the interaction between adaptive strategy and mulch type can enhance the success of perennial plant communities by optimising species compositions. Planning the management of established wildflower meadows can also be aided.

Key words: CSR strategies, landscape architecture, perennial meadow, sand garden, seed size, urban biodiversity.

Introduction

Wildflower meadows, which include both annual and perennial species, have become a popular trend in landscape design as an alternative model of greenspace that avoids low biodiversity of monoculture and block planting (Hitchmough 2004; Kuneva et al. 2008, 2009). Designing flower meadows as nature-like plant communities is a challenge for landscape architects and designers (Hitchmough 2017). Urban wildflower meadows have a positive impact on citizens (Hitchmough 2016). They are a strong tool for the mitigation of the urban island effect (Alizadeh and Hitchmough 2020). Ecologically based plant design is a way of reducing resources to maintain green areas (Hitchmough 2010).

Ecological theories have practical implications for the management of grasslands (Grime 1980, p. 21), and in this term could be useful for the management of established (artificial) wildflower meadows. Grime (1974, 1977, 2001) explains the CSR theory, which includes three main strategies that show how plants compete, deal with environmental limits, or recover from damage. Pierce et al.'s (2017) global method for calculating CSR strategy reveals the linkage between leaf traits and basic plant rates. This method and strategy–environment relationship helps to predict plant assemblage in response to changes in biogeochemical cycles, climate, and land use. The CSR model could assist in designing herbaceous communities in urban environments, selecting cultivar species, and predicting the effects of climate change (Wang et al. 2024b).

According to Grime (1980, p. 21), plant strategies can be understood as clusters of analogous genetic traits that consistently occur across various species or

populations, leading to recurring patterns in their ecological behaviour. He argues that in the context of grassland management, a functional understanding of these strategies may prove more beneficial than traditional taxonomic knowledge. Specifically, Grime (1980, p. 25) identifies three primary strategies: the competitive strategy, which is dominant in nutrient-rich and stable environments; the stress-tolerant strategy, associated with habitats characterized by persistent resource limitations; and the ruderal strategy, which prevails in ecosystems subject to frequent disturbance yet capable of high productivity. Furthermore, sources of ecological information are not only established grasslands but also recently disturbed areas that are in different phases of recolonization (Grime 1980, p. 14).

The CSR plant strategy of the dominant species is the primary factor that determines how habitat affects plant composition (Wang et al. 2024a). Some authors have investigated the strategies of natural plant communities and identified differences among various habitats (Dudova et al. 2019, Han et al. 2021, Wang et al. 2024a). CSR strategy theory based on leaf traits (Pierce et al. 2017) could be a useful tool in garden design for planning the right plants in certain environments (Wang et al. 2024b). For example, Fenzl and Kircher (2009) added to the work of Messer (2008) and Borchardt (1998) by suggesting five types of plant groups to use for mixing different perennial plants, focusing on their structure and main ways they compete. They are divided into structural plants (C, C-S, S), companion plants (C, C-S, S), groundcover (up to 30 cm) (C, C-S, S), filler (R, R-S, C-R), and scattered plants, which are described as 'plants with a short growth period that do not require much space'. These recommendations

are made for meadows established by plugs, eventually combined with sowing, but not only with sowing.

Management can be more economic and useful if it is informed by ecological methods and theories. The major aim of understanding ecological processes is to seek to manipulate them. (Grime 1980, p. 13). For example, rare mowing in productive habitats leads to 'expansion of large perennial grasses, tall forbs and shrubs' (Grime 1980, p. 22). Management during the peak of biomass, may be most effective for suppressing competitive plants. Also, reduction of productivity may be done by removing grass clippings.

There are two main factors that reduce plant biomass – stress and disturbance (Grime 1980, p. 22). Grime (1977) suggests that in environments with extremely low productivity, the ruderal stage may be entirely absent from the successional sequence, resulting instead in a gradual transition marked by the development of increasingly larger stress-tolerant plant forms. Accordingly, creating an environment for stress-tolerant plant species will decrease the abundance of competitive and ruderal plants, and also reduce the overall plant biomass. Since disturbances do not correlate with stress-tolerant plants, the mowing regime must be reduced (Grime 1980, p. 31).

In this context, designing an unproductive wildflower meadow within an urban environment offers economic advantages. Less biomass means less expenses for removal and disposal of mown parts (Grime 1980, p. 31; Hoyle et al. 2017). However, creating harsh conditions for stress-tolerant plants may result in an overall reduction in seed germination.

Seed mass and other seed production traits can affect plant survival independently of primary strategy (Pierce et

al. 2014). Seed size has a significant influence on the seedling survival and height growth rate. Larger seeds have a significantly higher chance to survive (Fang et al. 2013). Large seeds, which are over 5.0 mm, demonstrate significantly higher seedling germination than intermediate (2.0–5.0 mm) or small seeds (< 2.0 mm). Larger seeds absorb 100 times more water than small seeds (Hitchmough and de la Fleur 2006, Hitchmough et al. 2008, Fang et al. 2013).

Soil parameters primarily align shifts in CSR plant strategies among different environments. Soil and climate variables significantly correlate with the dominant strategy (Rafiee et al. 2024). Mulching is a widely used technique in landscape design changes soil characteristic. Water retaining is one of the most important characteristics of the mulch. The other is inhibiting weed germination. There are some techniques for decreasing weeds from soil seed bank. Removing the top 15 cm of soil and applying 5 cm of sand mulch before sowing greatly reduces weed competition in the first growing season (Gao et al. 2012, Bretzel et al. 2024). Applying 7.5 cm of mulch sand while removing only the existing vegetation also has a positive impact (Hitchmough 2017, Hristova 2024b).

However, organic mulch has a negative impact on species richness (Hristova 2024b). It suppresses seeds in two ways: mechanically – through the thickness of the mulch layer – and physiologically – by releasing phytotoxins, which act as growth inhibitors (Waldron and Nichols 2009). The quantitative ratio of carbon to nitrogen leads to the formation of different compounds. Higher carbon levels lead to organic acids, while higher nitrogen levels lead to ammonia. Both, in high amounts, inhibit seed germination (Waldron and

Nichols 2009, Acharya et al. 2018). When mulch is used for sowing, it alters the germination environment in the topsoil layer where it is applied. In addition to modifying soil conditions, mulch can influence plant growth and species establishment.

The aim of this study is to explore how different mulching substrates influence early stages of plant community assembly. Specifically, it examines two main aspects: first, how species' CSR (competitor–stress-tolerator–ruderal) strategies relate to resulting species composition; and second, whether seed size affects initial germination success. In addition, the study considers which adaptive strategies are associated with species that become dominant under particular mulch conditions.

Material and Methods

Experiment establishment and field studies

The experimental plots were established at the Vrazhdebna Educational and Experimental Field, located in the northeastern part of Sofia, Bulgaria (42°42'26.4" N, 23°26'12.0" E) in a moderate continental climate with highest precipitation in May, June, and July (Koleva and Peneva 1990). The soil is mostly sandy with a low quantity of loam, rich in phosphorus and with neutral acidity. Climatic and soil conditions at the site are described in detail in Hristova (2024a).

First, the soil was manipulated with a rotary tiller, and all plant parts were removed. Two types of mulch were applied over the topsoil in a 75 mm layer: mineral mulch (sand, 0–4 mm fraction) and organic mulch (compost processed at Sofia's MBT municipal waste plant 'Han Bogrov'

facility, 0–10 mm fraction; C: N = 13.7, pH = 7.06, water content 42.5 %, moisture 657.9 g·L⁻¹ and 100% germination coefficient according to the Institute for Sustainable Plant Production 2022). A control condition without mulch was also included. Each mulch type was assigned to three replicate plots 1.5 × 1.5 m, each containing a different seed mixture. Seed mixtures are based on semi-natural habitats used for pastures or hay meadows, usually the alliance *Arrhenatherion elatioris* Koch (1926). The seeds are used for landscape design. Both economic and aesthetic factors influence their production (Mainz and Wieden 2019). Restoration projects, which are more specific tasks than general landscape design projects, are not the focus of this research.

In early December 2021, seeds from perennial herbaceous plants – previously used in an earlier experiment (Hristova 2024a, 2024b) – were sown in the experimental plots (Appendix A, Table A1). Due to an initial period of drought followed by prolonged rainfall, the seeds were sown during winter under suboptimal conditions, specifically onto slightly waterlogged soil that remained overly moist at the surface. They were applied over the mulching substrates, raked and compacted with a plank. No additional irrigation was provided after sowing.

During the first growing season, all plots were weeded, although some annual weeds with ornamental value (e.g., *Consolida regalis* Gray) were left intact. In June of the third year, two assessments were conducted to measure the projected cover of each species in every plot.

Plant identification was done according to Yordanov (1963), Fetovadzhieva (1973), and Delipavlov et al. (2011). Non-native species were identified based on seed mix specifications.

Data analysis

'A field-portable list of CSR strategies' (Pierce et al. 2017) was used for determining the adaptive strategies of the species. If some species were not presented, the nearest taxonomic species values were used. To assess relative importance, the methods from Wang et al. (2024a) were applied. The importance value (I_v) and dominance (D) were calculated using the formulas from Ohsawa (1984) – formulas (1) and (2), respectively.

$$I_v = H \cdot C, \quad (1)$$

$$D = \frac{I_v}{\sum (I_v \cdot 100)}, \quad (2)$$

where: I_v is the species' importance; H is the maximum height, m; C is the species' projected cover; and D is the value of dominance of a species within a plot.

The relative importance (RI) of a species was classified into five categories: dominant species ($D \geq 20$), subdominant species ($10 \leq D < 20$), companion species ($5 \leq D < 10$), rare species ($1 \leq D < 5$), and extremely rare species ($D < 1$).

The C-S-R scores were classified into 19 groups based on the dominant strategy (Hodgson 1999). The community weighted means (CWMs) for each plot were calculated using the dominance (D) to compute a weighted average.

Descriptive statistics were calculated for the weighted mean scores of C (competitor), S (stress-tolerator), and R (ruderal) strategies across the three mulch types.

The influence of mulch type and seed size on germination was examined. The maximum species height and air-dry weight of 1000 seeds were extracted from the published 'LEDA traitbase' (Kleyer et

al. 2008). Seed size is defined by seed mass of 1000 air-dry seeds (M), which was categorised as follows: $0 \text{ g} \leq M < 1 \text{ g}$ (small), $1 \text{ g} \leq M < 5 \text{ g}$ (medium), and $M \geq 5 \text{ g}$ (large).

For each RI and CSR class, species from the seed mixes that were found in the plots were counted; if a species was not found, it was assigned a value of 0. The Shapiro-Wilk test show that the data was not normally distributed. There are too many zeros for conducting ANOVA. Therefore, a zero-inflated negative binomial (ZINB) regression was conducted to examine the effects of mulch, relative importance (RI) and CSR strategies, including their interactions on the quantity of species in each group.

Binary logistic regression was applied to analyse the effect of mulch substrates and seed weight on germination success and their interactions. Since the response variable (germination) is binary – coded as 1 if the species germinated, and 0 if it did not – logistic regression is an appropriate method for modelling the probability of germination based on categorical and continuous predictors.

The zero-inflated negative binomial (ZINB) regression and the binary logistic regression were conducted using the R package pscl (Jackman 2024) and ggplot2 (Wickham 2016). The R package dplyr (Wickham et al. 2023) was used for means and standard deviations. Also 'ggtern' (Hamilton and Ferry 2018) an extension to ggplot2, for the creation of ternary diagrams.

Dispersion was calculated using the sum of squared Pearson residuals divided by the residual degrees of freedom in the generalized linear model.

Results

CWMs of C-, S-, R-scores in different mulch types

In sand mulch, the C strategy showed a mean of 31.00 (SD = 2.35), the S strategy had a mean of 25.30 (SD = 5.13), and the R strategy had the highest mean at 43.70 (SD = 7.48). There was a lowest dispersion for C- and S-scores in the sand (figs 1 and 2).

For control plots (bare soil), the C strategy has the lowest mean ($M = 25.50$, $SD = 11.60$), while the S strategy was higher ($M = 34.80$, $SD = 9.35$), and the R strategy remained relatively high ($M = 39.70$, $SD = 2.75$) (figs 1 and 2).

In compost, the C strategy reached its highest mean ($M = 35.10$, $SD = 12.90$), the S strategy dropped to its lowest ($M = 18.10$, $SD = 13.90$), and the R strategy had the lowest mean ($M = 46.90$, $SD = 24.10$) but the highest variation and the highest R scores (figs 1 and 2).

CSR strategy of dominant species

A zero-inflated negative binomial (ZINB) regression showed that the number of species in each RI group was noticeably different ($SE = 0.28$, $p < 0.001$), and a higher RI score was associated with fewer species. Furthermore, there was significant interaction between RI groups and C-, S-, and R- groups ($SE = 0.02$, $p = 0.045$). Mulch ($SE = 0.44$, $p = 0.098$) and CSR ($SE = 0.09$, $p = 0.103$) showed trends but were not significant main effects. The model showed a satisfactory fit (dispersion = 1.34) (Table 1).

In sand mulch, species predominantly exhibited ruderal strategies. The sandy substrate, which is characterised by low nutrient content and poor water retention, favours plants that invest in rapid growth and have high reproductive outputs. Competitive and stress-tolerant traits were expressed to a lesser extent, suggesting that survival in this environment depends more on rapid colonisation than long-term

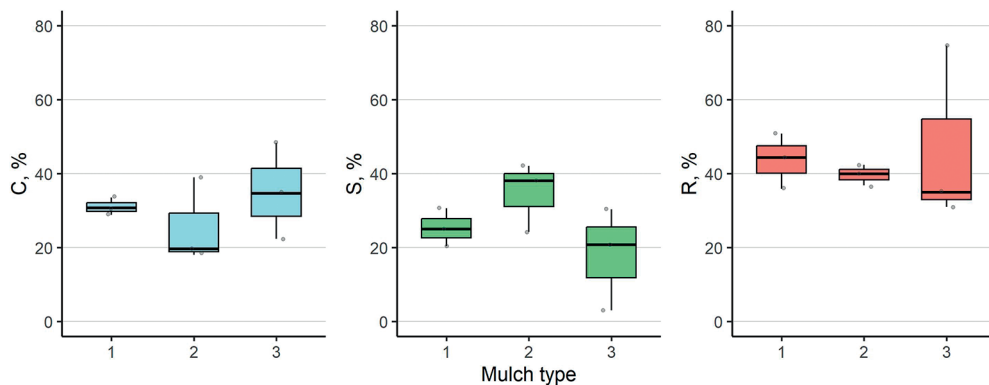


Fig. 1. Mean values and standard deviations of community-weighted means (CWMs) of C-, S-, and R-strategy values across different mulch types.

Note: 1 – sand, 2 – control (bare soil), 3 – compost.

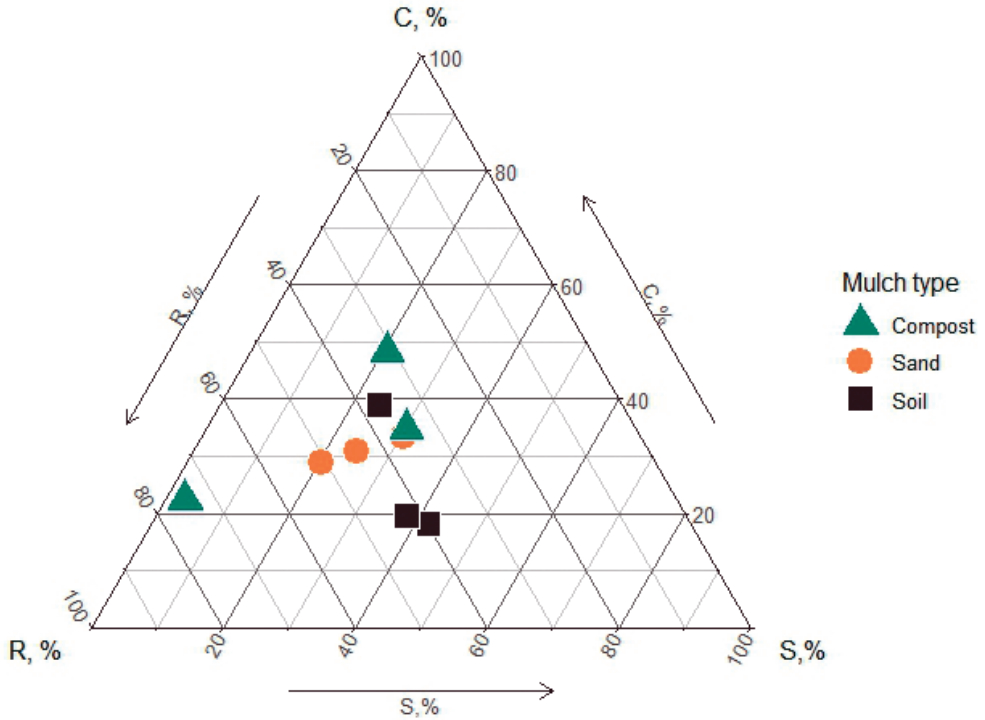


Fig. 2. Community weighted means (CWMs) of C-, S-, and R-scores in sand, bare soil and compost.

Table 1. Count model coefficients (ZINB).

Predictor	Estimate	Std. Error	p-value
Intercept	1.714	1.128	0.129
Mulch type	-0.725	0.438	0.098
RI	-1.087	0.278	<0.001 ***
CSR strategy	-0.141	0.086	0.103
Mulch type × CSR strategy	0.045	0.034	0.191
RI × CSR strategy	0.040	0.020	0.045 *

Note: ZINB model; dispersion = 1.34, * $p < 0.05$, *** $p < 0.001$.

resource monopolisation or physiological resilience (Fig. 3).

There were dominant species, including *Centaurea jacea* L., *Leucanthemum vulgare* Lam. (C:S:R = 20.85:0:79.15 %),

Festuca trichophylla (Duval-Jouve) C.E.Hubb. (C:S:R = 10.41:35.7:53.89 %), *Sanguisorba minor* Scop. (C:S:R = 25.53:43.98:30.48 %) and *Anthemis tinctoria* L. (C:S:R = 15.6:43.45:40.95 %) (Table 2).

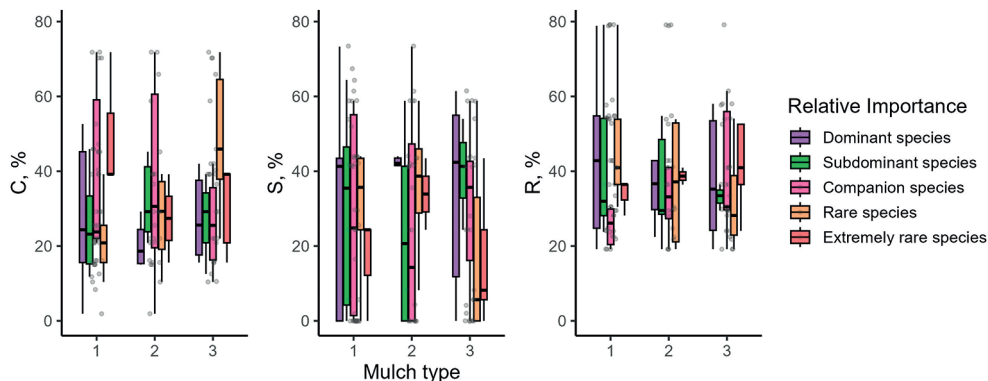


Fig. 3. C-, S-, R-scores of RI groups across different mulch types: 1 – sand, 2 – bare soil, 3 – compost.

Table 2. Dominant species and their C-, S-, R-strategy values among different mulch types.

Species	Tertiary CSR strategy	C-, S-, R-strategy values			Mulch type		
		C, %	S, %	R, %	Sand	Soil	Com-post
<i>Achillea millefolium</i> L.	S/CSR	22.02	58.81	19.18			+
<i>Anthemis tinctoria</i> L.	SR/CSR	15.60	43.45	40.95	+	+	
<i>Centaurea jacea</i> L.	CR/CSR	39.19	24.35	36.46	+	+	+
<i>Cichorium intybus</i> L.	CS/CSR	36.55	41.68	21.77			+
<i>Consolida regalis</i> Gray	SR/CSR	20.39	36.93	42.68		+	
<i>Festuca trichophylla</i> (Duval-Jouve) C.E.Hubb.	SR	10.41	35.70	53.89	+		
<i>Knautia arvensis</i> (L.) Coult.	C/CR	70.27	5.66	24.07			+
<i>Leucanthemum vulgare</i> Lam.	R/CR	20.85	0	79.15	+		+
<i>Sanguisorba minor</i> Scop.	S/CSR	25.53	43.98	30.48	+		

In soil without mulch, species tend to adopt stress-tolerant strategies. Soil provides more stable but moderately challenging environment, possibly due to variations in moisture, temperature, or other stress factors. In this context, species prioritise traits that support resilience and structural persistence. Competitive and ruderal traits were still present but not as dominant, indicating a balanced strategic profile shaped by moderate abiotic stress (Fig. 3).

Anthemis tinctoria (C:S:R = 15.6:43.45:40.95 %) and *Centaurea jacea* (C:S:R = 39.19:24.35:36.46 %) were dominant species in control plots. *Consolida regalis* (C:S:R = 20.39:36.93:42.68 %) was also dominant in the same plots (Table 2).

In compost mulch, species show a mix of competitive and ruderal strategies. The high nutrient availability and improved moisture conditions promote both resource acquisition and rapid growth. This environment supports a wider vari-

ety of strategic behaviours, allowing both dominant competitors and opportunistic colonisers to thrive. Stress tolerance is less pronounced, reflecting the relatively benign conditions created by the compost substrate (Fig. 3).

There were also more than one dominant species in compost: *Centaurea jacea*, *Cichorium intybus* L. (C:S:R = 36.55:41.68:21.77 %), *Knautia arvensis* (L.) Coult. (C:S:R = 70.27:5.66:24.07 %), *Achillea millefolium* L. (C:S:R = 22.02:58.81:19.18 %), and *Leucanthemum vulgare* (C:S:R = 20.85:0:79.15 %). *Centaurea jacea* was a dominant species in all mulch types (Table 2). Appendix B (Fig. B1 and Table B1) presents the remaining RI groups across substrates.

The influence of a mulch type and seed size on germination

The overall binary logistic regression model was statistically significant, with a residual deviance of 665.29. A chi-square test was performed to evaluate whether the inclusion of eight predictors significantly improved the model's fit compared to the null model. The test yielded a chi-square value of 25.97 with 8 degrees of freedom, resulting in a p -value below 0.05 (χ^2 (8)

= 25.97, $p < 0.05$). The model demonstrated an acceptable fit, as reflected by the Akaike Information Criterion (AIC = 683.29) and a dispersion parameter close to 1 (dispersion = 1.02) (Table 3).

A significant negative main effect was observed for compost (SE = 0.34, $p = 0.014$), suggesting lower odds of germination under compost compared to the sand and control plots (bare soil). The interaction between bare soil and large seeds was also statistically significant (SE = 1.14, $p = 0.029$), indicating a negative effect of bare soil over large seeds. There is also significant negative effect of interception between sand mulch and small seeds (SE = 0.22, $p = 0.02$). No other main effects or interactions reached significance ($p > 0.05$) (Table 3).

Discussion

CSR strategy

There was a notable difference in the CSR strategies of plants across various relative importance groups. Interactions between CSR groups and mulches indicated a trend; however, it is likely that larger plots are required for more definitive results.

Table 3. GLM Coefficients for effect of seed size on germinated specie in different mulch types.

Predictor	Estimate	SE	p -value
Intercept	-0.511	0.220	0.020*
Control plots	-0.413	0.323	0.201
Compost	-0.847	0.344	0.014*
Medium seeds	-0.251	0.318	0.429
Large seeds	0.201	0.454	0.658
Control plots × Medium seeds	-0.183	0.476	0.701
Compost × Medium seeds	0.251	0.491	0.608
Control plots × Large seeds	-2.495	1.141	0.029*
Compost × Large seeds	-0.547	0.756	0.469

Note: dispersion = 1.02, * $p < 0.05$ indicates statistical significance.

Both the type of mulch and the adaptive strategy affected plant composition. The relative importance groups exhibited varying means of C-, S-, and R-scores across different substrates.

The results align with those of Wang et al. (2024a) and reaffirm the connection between the dominant species primary strategy and the characteristics of the community. Understanding the primary strategy of dominant species sheds light on environmental conditions.

In sandy habitats dominated by ruderal (R) species, mowing should be carefully timed to coincide with periods of sufficient soil moisture. Given the low water-holding capacity of sandy substrates, moisture availability is critical for the rapid regeneration and reproductive success characteristic of R-strategists. Extreme stress and/or disturbance create an environment in which few species can survive (Grime 1980, p. 33). Mowing during dry periods can inhibit regrowth, reduce vegetative cover, ultimately undermining the persistence of ruderal species. Therefore, for management practices aiming to maintain or promote R-dominated plant communities in sandy environments, mowing is most effective when conducted during or immediately following rainfall events or within wetter seasons that support rapid post-disturbance recovery.

In bare soil, where stress-tolerant species tend to dominate, mowing should be applied with caution and at a low frequency. These species typically invest in structural stability and long-term persistence rather than rapid regrowth, making them less responsive to frequent disturbance. Intensive or poorly timed mowing may disrupt the equilibrium of such communities, reduce biomass recovery, and favour the encroachment of more ruderal or opportunistic species. To maintain the functional

integrity of stress-adapted assemblages, mowing should be limited to occasional interventions, preferably during periods of adequate soil moisture and minimal physiological stress. Low-frequency mowing – such as once per year – can benefit S-R strategists, which are adapted to moderately disturbed, unproductive habitats (Grime 1980, p. 25).

In compost-enriched environments, where both competitive and ruderal strategies are expressed, mowing should be applied at moderate frequency and timed to balance growth control with species regeneration. The high nutrient and moisture availability in compost supports rapid biomass accumulation, which, if unmanaged, can lead to competitive exclusion. The results are in agreement with the findings of Sparke et al. (2011). More than 45 % organic matter creates too fertile conditions and increases weeds. Periodic mowing – conducted two to three times per growing season and aligned with phases of active growth (Grime 1980, p. 22) – can help regulate dominant competitors while allowing ruderal species to complete their life cycles. Such a regime supports strategic diversity and maintains a dynamic, yet stable, community structure.

Seed size

Although large seeds are typically more resilient to stressful environments due to their greater energy reserves, their germination was significantly reduced under bare soil conditions. This finding contrasts with expectations based on seed size and their stress tolerance but aligns with their advantage in litter cover (Lönnerberg and Eriksson 2013, Muller-Landau 2010). Compared to sand, the probability of germination for large-seeded species was approximately 2.5 times lower in compost

and more than 11 times lower in control plots (bare soil), the latter being statistically significant. These results suggest that, under certain mulch-free conditions, even stress-tolerant traits like large seed size may not compensate for the harsher surface microclimate or soil compaction that may inhibit germination. In parallel, although large seeds typically absorb water up to 100 times faster than small seeds (Osmond et al. 1980) and generally exhibit higher germination rates in the field, excess soil moisture during winter may have triggered premature germination or caused seed damage through freezing.

Meanwhile, under sand mulch, small-seeded species were significantly more frequent. Compared to sand, the number of small-seeded species was approximately 1.5 times lower in bare soil and 2.3 times lower in compost. The harsh abiotic conditions at the soil surface – such as poor water retention – likely reduce the survival chances of both small-seeded species and slow-establishing large-seeded species, highlighting the dual vulnerability of extreme seed sizes under suboptimal mulch conditions. The highest number of small-seeded species successfully germinated in sand, suggesting that the loose, well-drained structure of this substrate provided more favourable microsite for light-dependent and surface-germinating species.

These findings are consistent with those of Hitchmough and de la Fleur (2006), who reported that sand mulch plots positively affect germination of prairie vegetation. Seed size influences germination; however, larger seeds did not exhibit a clear advantage across all substrates. This outcome diverges from the results presented by Hitchmough et al. (2008) and Fang et al. (2013) but species with large seeds are the most in sand

mulch plots. In bare soil, the lack of mulch likely led to surface crust formation and faster evaporation, which are especially detrimental to larger seeds that require more consistent moisture and stable conditions for germination. In contrast, both compost and sand mulches may have mitigated these stresses by improving surface structure, maintaining humidity, and reducing soil compaction.

The germination index of the experimental compost was 100 % (Institute for Sustainable Plant Production 2022), which mean that the compost is mature. However, regardless of seed size, compost mulch suppressed overall germination of the target plants. The nitrogen content in compost was a normal to slightly lower, with a C: N ratio of 13.7. By comparison, soils in Central and Eastern Europe typically exhibit C:N ratios in the range of 9–12 (Batjes 2002). The nitrogen content was much higher than the usual compost range of 20.4–40.1 (Enakiev et al. 2017, 2022). The optimal C:N ratio is between 25 and 30 (Haug 1980). Low C:N ratios under 15 can lead to the production of ammonia, a known inhibitor of seed germination (Haug 1993, Waldron and Nichols 2009). Compost with C:N = 15 has lower germination index (53–66 %) in comparison with 18 and 21. The recommended parameters for composting are an aeration rates of $0.48 \text{ L} \cdot (\text{kg DM})^{-1} \cdot \text{min}^{-1}$ and a C:N ratio of 18 with moisture contents of 65–75 % (Guo et al. 2012). While compost mulch can improve surface conditions by retaining moisture, its loose structure and lightness may also make it prone to displacement by wind or rainfall. This instability can expose germinating seeds to drying conditions or temperature fluctuations. This suggests that while mulch generally creates more favourable micro-environment for seed emergence, its spe-

cific physical properties – such as structural stability – also influence germination success. Another possible reason is the presence of soil-borne fungal or bacterial pathogens, which often grow well in nutrient-rich, organic materials and can greatly harm seed survival and seedling growth.

These results have practical implications for the design of flowering meadows, particularly in selecting appropriate mulching materials, composing effective seed mixtures, and optimizing the management of established meadows. Further experimental work is needed to better understand the underlying mechanisms and to evaluate germination outcomes across different compost formulations. Future research should also consider calculating CSR scores using leaf traits measured directly from experimental plants to improve the accuracy of strategy classification.

Conclusion

The study demonstrates that mulch type influences plant composition by shaping the dominance of different ecological strategies and affecting seed germination. Sand mulch favours species with ruderal strategy, adapted to frequent disturbances. Supporting their regeneration requires carefully timed mowing during moist periods. Under winter sowing conditions, large seeds perform well in sand mulch, but small seeds show significantly better performance in sand compared to other substrates. Bare soil significantly reduces the germination of large-seeded species and favours stress-tolerant plants that are sensitive to disturbance. Implementing low-frequency mowing may help maintain a balance among different ecological strategies. Compost significantly reduces germination regardless of seed size.

Competitive species tend to perform best in compost mulch, suggesting that more frequent mowing may be necessary to prevent their dominance and maintain species diversity. These findings highlight the importance of tailoring management practices, such as mowing regimes and mulch selection, to both abiotic conditions and the functional traits of target plant communities.

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Appendix A

Table A1. Plant species in seed mixes.

No	Species	Seed mixes		
		02	06	06a
1	<i>Achillea millefolium</i> L.	+	+	+
2	<i>Agrimonia eupatoria</i> L.		+	
3	<i>Anthemis tinctoria</i> L.		+	+
4	<i>Anthoxanthum odoratum</i> L.		+	+
5	<i>Anthriscus sylvestris</i> (L.) Hoffm.	+		
6	<i>Anthyllis vulneraria</i> L.		+	+
7	<i>Arrhenatherum elatius</i> (L.) P.Beauv.	+		
8	<i>Bellis perennis</i> L.	+		
9	<i>Briza media</i> L.			+
10	<i>Bromus erectus</i> Huds.			+
11	<i>Bupthalmum salicifolium</i> L.		+	+
12	<i>Campanula patula</i> L.	+	+	+
13	<i>Campanula rotundifolia</i> L.			+
14	<i>Carum carvi</i> L.		+	
15	<i>Centaurea jacea</i> L.	+	+	+
16	<i>Centaurea scabiosa</i> L.		+	+
17	<i>Cichorium intybus</i> L.		+	
18	<i>Crepis biennis</i> L.	+	+	
19	<i>Cynosurus cristatus</i> L.	+	+	+
20	<i>Daucus carota</i> L.	+	+	+
21	<i>Dianthus carthusianorum</i> L.		+	+
22	<i>Echium vulgare</i> L.		+	
23	<i>Festuca ovina</i> L.			+
24	<i>Festuca trichophylla</i> (Duval-Jouve) C.E.Hubb.			+
25	<i>Galium mollugo</i> L.	+	+	
26	<i>Galium verum</i> L.		+	+
27	<i>Geranium pratense</i> L.		+	
28	<i>Heracleum sphondylium</i> L.	+		
29	<i>Hypericum perforatum</i> L.		+	+
30	<i>Knautia arvensis</i> (L.) Coult.	+	+	+
31	<i>Leontodon hispidus</i> L.	+		+
32	<i>Leucanthemum vulgare</i> Lam.	+	+	+
33	<i>Lotus corniculatus</i> L.		+	+
34	<i>Malva moschata</i> L.		+	+
35	<i>Medicago lupulina</i> L.		+	+
36	<i>Onobrychis viciifolia</i> Scop.	+	+	+
37	<i>Picris hieracioides</i> L.	+		
38	<i>Pimpinella saxifraga</i> L.		+	+

No	Species	Seed mixes		
		02	06	06a
39	<i>Plantago lanceolata</i> L.	+	+	
40	<i>Potentilla recta</i> L.		+	
41	<i>Prunella grandiflora</i> (L.) Scholler	+	+	+
42	<i>Prunella vulgaris</i> L.	+	+	
43	<i>Ranunculus acris</i> L.	+		
44	<i>Ranunculus bulbosus</i> L.	+		+
45	<i>Rhinanthus alectorolophus</i> (Scop.) Pollich		+	+
46	<i>Rumex acetosa</i> L.	+		
47	<i>Salvia pratensis</i> L.	+	+	+
48	<i>Salvia verticillata</i> L.		+	
49	<i>Sanguisorba minor</i> Scop.	+	+	+
50	<i>Scabiosa columbaria</i> L.			+
51	<i>Silene nutans</i> L.		+	
52	<i>Silene vulgaris</i> (Moench) Garcke	+	+	+
53	<i>Thymus pulegioides</i> L.			+
54	<i>Tragopogon orientalis</i> Willd.		+	+
55	<i>Trisetum flavescens</i> (L.) P.Beauv.	+	+	
56	<i>Veronica teucrium</i> L.			+

Note: 02 – wet meadow seed mix, 06 – universal meadow seed mix, 06a – dry meadow seed mix.

Source: (Hristova 2024a).

Appendix B

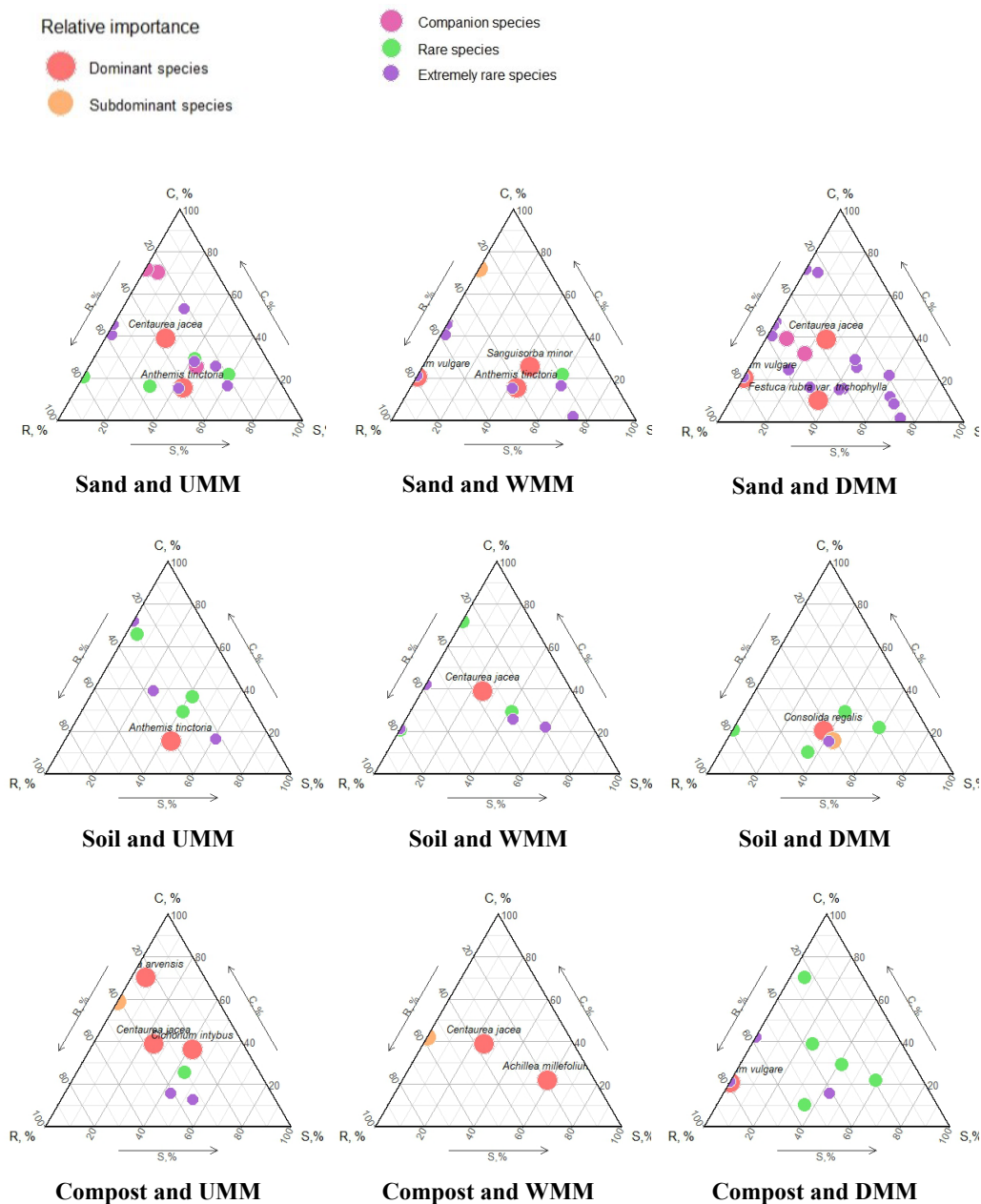


Fig. B1. C-, S-, R-strategy values and relative importance of species in each plot.
Note: UMM – universal meadow mix, WMM – wet meadow mix, DMM – dry meadow mix.

Table B1. Relative importance and C-, S-, R-scores of indicated plants.

No	Species	C, %	S, %	R, %	Relative importance
Soil + Universal meadow mix (06)					
1	<i>Anthemis tinctoria</i> L.	15.6	43.45	40.95	5
2	<i>Cichorium intybus</i> L.	36.55	41.68	21.77	2
3	<i>Echium vulgare</i> L.	65.91	4.25	29.84	2
4	<i>Daucus carota</i> L.	29.20	41.31	29.49	2
5	<i>Centaurea jacea</i> L.	39.19	24.35	36.46	1
6	<i>Plantago lanceolata</i> L.	71.82	0	28.18	1
7	<i>Dianthus carthusianorum</i> L.	16.15	61.42	22.44	1
Soil + Wet meadow mix (02)					
1	<i>Centaurea jacea</i>	39.19	24.35	36.46	5
2	<i>Leucanthemum vulgare</i> Lam.	20.85	0	79.15	2
3	<i>Plantago lanceolata</i>	71.82	0	28.18	2
4	<i>Daucus carota</i>	29.20	41.31	29.49	2
5	<i>Achillea millefolium</i> L.	22.02	58.81	19.18	1
6	<i>Bellis perennis</i> L.	21.13	0	78.87	1
7	<i>Sanguisorba minor</i> Scop.	25.53	43.98	30.48	1
8	<i>Tragopogon orientalis</i> Willd.	42.00	0	58.00	1
Soil + Dry meadow mix (06a)					
1	<i>Consolida regalis</i> Gray	20.39	36.93	42.68	5
3	<i>Anthemis tinctoria</i>	15.6	43.45	40.95	4
4	<i>Achillea millefolium</i>	22.02	58.81	19.18	2
5	<i>Daucus carota</i>	29.2	41.31	29.49	2
2	<i>Festuca trichophylla</i> (Duval-Jouve) C.E.Hubb.	10.41	35.7	53.89	2
6	<i>Leucanthemum vulgare</i>	20.85	0	79.15	2
7	<i>Cynosurus cristatus</i> L.	15.05	42.12	42.83	1
Sand + Universal meadow mix (06)					
1	<i>Centaurea jacea</i>	39.19	24.35	36.46	5
2	<i>Anthemis tinctoria</i>	15.6	43.45	40.95	5
3	<i>Knautia arvensis</i> (L.) Coult.	70.27	5.66	24.07	3
4	<i>Plantago lanceolata</i>	71.82	0	28.18	3
5	<i>Sanguisorba minor</i>	25.53	43.98	30.48	3
6	<i>Achillea millefolium</i>	22.02	58.81	19.18	2
7	<i>Daucus carota</i>	29.20	41.31	29.49	2
8	<i>Leucanthemum vulgare</i>	20.85	0	79.15	2
9	<i>Medicago lupulina</i> L.	16.11	29.63	54.26	2
10	<i>Dianthus carthusianorum</i>	16.15	61.42	22.44	1
11	<i>Potentilla recta</i> L.	25.56	51.8	22.64	1
12	<i>Salvia verticillata</i> L.	52.62	25.51	21.87	1
13	<i>Silene nutans</i> L.	40.36	1.98	57.66	1
14	<i>Silene vulgaris</i> (Moench) Garcke	45.20	0	54.80	1
15	<i>Cynosurus cristatus</i>	15.05	42.12	42.83	1
16	<i>Scabiosa columbaria</i> L.	27.83	42.09	30.08	1

No	Species	C, %	S, %	R, %	Relative importance
Sand + Wet meadow mix (02)					
1	<i>Sanguisorba minor</i>	25.53	43.98	30.48	5
2	<i>Anthemis tinctoria</i>	15.60	43.45	40.95	5
3	<i>Leucanthemum vulgare</i>	20.85	0	79.15	5
4	<i>Plantago lanceolata</i>	71.82	0	28.18	4
5	<i>Achillea millefolium</i>	22.02	58.81	19.18	2
6	<i>Leontodon hispidus</i> L.	45.93	0	54.07	1
7	<i>Bellis perennis</i>	21.13	0	78.87	1
8	<i>Silene vulgaris</i>	45.20	0	54.8	1
9	<i>Cynosurus cristatus</i>	15.05	42.12	42.83	1
10	<i>Dianthus carthusianorum</i>	16.15	61.42	22.44	1
11	<i>Silene nutans</i>	40.36	1.98	57.66	1
12	<i>Festuca ovina</i> L.	1.91	73.32	24.77	1
Sand + Dry meadow mix (06a)					
1	<i>Centaurea jacea</i>	39.19	24.35	36.46	5
2	<i>Leucanthemum vulgare</i>	20.85	0	79.15	5
3	<i>Festuca trichophylla</i>	10.41	35.7	53.89	5
4	<i>Stachys recta</i> L.	32.14	19.12	48.74	3
5	<i>Picris hieracioides</i> L.	39.24	8.20	52.56	3
6	<i>Knautia arvensis</i>	70.27	5.66	24.07	1
7	<i>Sanguisorba minor</i>	25.53	43.98	30.48	1
8	<i>Bromus erectus</i> Huds.	11.81	64.39	23.8	1
9	<i>Achillea millefolium</i>	22.02	58.81	19.18	1
10	<i>Anthemis tinctoria</i>	15.60	43.45	40.95	1
11	<i>Buphthalmum salicifolium</i> L.	24.35	16.62	59.03	1
12	<i>Daucus carota</i>	29.2	41.31	29.49	1
13	<i>Leontodon hispidus</i>	45.93	0	54.07	1
14	<i>Medicago lupulina</i>	16.11	29.63	54.26	1
15	<i>Salvia pratensis</i> L.	47.24	0	52.76	1
16	<i>Silene vulgaris</i>	45.20	0	54.80	1
17	<i>Anthoxanthum odoratum</i> L.	8.36	67.45	24.19	1
18	<i>Cynosurus cristatus</i>	15.05	42.12	42.83	1
19	<i>Festuca ovina</i>	1.91	73.32	24.77	1
20	<i>Silene nutans</i>	40.36	1.98	57.66	1
21	<i>Bellis perennis</i>	21.13	0	78.87	1
22	<i>Plantago lanceolata</i>	71.82	0	28.18	1
Compost + Universal meadow mix (06)					
1	<i>Centaurea jacea</i>	39.19	24.35	36.46	5
2	<i>Cichorium intybus</i>	36.55	41.68	21.77	5
3	<i>Knautia arvensis</i>	70.27	5.66	24.07	5
4	<i>Crepis biennis</i> L.	58.80	0	41.20	4
5	<i>Sanguisorba minor</i>	25.53	43.98	30.48	2
6	<i>Prunella vulgaris</i> L.	12.55	53.99	33.47	1
7	<i>Anthemis tinctoria</i>	15.6	43.45	40.95	1

No	Species	C, %	S, %	R, %	Relative importance
Compost + Wet meadow mix (02)					
1	<i>Achillea millefolium</i>	22.02	58.81	19.18	5
2	<i>Centaurea jacea</i>	39.19	24.35	36.46	5
3	<i>Tragopogon orientalis</i>	42.00	0	58.00	4
Compost + Dry meadow mix (06a)					
1	<i>Leucanthemum vulgare</i>	20.85	0	79.15	5
2	<i>Knautia arvensis</i>	70.27	5.66	24.07	2
3	<i>Centaurea jacea</i>	39.19	24.35	36.46	2
4	<i>Festuca trichophylla</i>	10.41	35.70	53.89	2
5	<i>Achillea millefolium</i>	22.02	58.81	19.18	2
6	<i>Daucus carota</i>	29.20	41.31	29.49	2
7	<i>Anthemis tinctoria</i>	15.60	43.45	40.95	1
8	<i>Tragopogon orientalis</i>	42.00	0	58.00	1
9	<i>Bellis perennis</i>	21.13	0	78.87	1

Acclimation changes in photosynthesis of *Fagus sylvatica*, *Populus tremula*, and *Acer platanoides* after natural disturbance

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Thematic field: Climate change and forests

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Abstract

Large-scale natural disturbances in forest ecosystems drastically alter the light regime for residual saplings within the affected areas, placing their survival under considerable threat. Light acclimation of photosynthesis represents a key physiological mechanism for maintaining a positive carbon balance, thereby enabling plants to preserve their protective functions within altered environments. The aim of this study was to assess interspecific differences in photosynthetic light acclimation and carbon-use efficiency in saplings of three forest tree species – *Fagus sylvatica* L., *Populus tremula* L. and *Acer platanoides* L. – following large-scale natural disturbances. These species were selected due to their contrasting ecological strategies: the shade-tolerant *F. sylvatica*, the light-demanding pioneer *P. tremula*, and the moderately plastic *A. platanoides*. A comparative analysis of parameters, such as A_{MAX} , R_D , LCP, and LSP reveals pronounced species-specific responses, reflecting divergent functional–ecological strategies. These findings provide deeper understanding of the mechanisms underpinning light acclimation and offer valuable insights for adaptive forest management, particularly considering the increasing frequency and intensity of climate-induced disturbances. The results may support improved predictions of regeneration dynamics and species resilience under future forest conditions.

Key words: carbon balance, light-response curve, photosynthetic plasticity.

Introduction

Temperate forest ecosystems have been facing significant challenges arising from the intensifying impacts of climate change. In recent decades, there has

been a marked increase in the frequency and severity of natural disturbances, such as windthrows, wildfires, and droughts, which have led to abrupt alterations to microclimatic conditions within affected areas, including the light regime, particularly

in the understorey layer (Seidl et al. 2017). Such disturbances result in the removal of substantial portions of the forest canopy, allowing intense light to penetrate to the regenerating saplings below. The sudden change in light availability for these young trees represents both a potential threat and an opportunity for growth, provided they possess effective mechanisms for acclimation.

Light acclimation of photosynthesis plays a pivotal role in enabling plants to sustain a positive carbon balance, which is critical not only for growth but also for the activation of metabolic pathways associated with defence and recovery following stress (Valladares and Niinemets 2008). Plants exhibit species-specific strategies in response to light stress, ranging from rapid physiological adjustments to structural modifications in leaf morphology and photosynthetic apparatus (Niinemets and Valladares 2006).

Fagus sylvatica L., as a shade-tolerant species, demonstrates limited plasticity, yet its capacity to maintain stable photosynthetic activity under low irradiance confers it with a competitive advantage in dense, closed-canopy forests. A study by Marinova and Anev (2024) has confirmed that small canopy openings created through carefully planned group selection cutting provide more favourable conditions for the growth of young beech saplings than large canopy destruction. Under such conditions, these individuals are able to develop more successfully without experiencing severe physiological stress or loss of leaf area, indicating that light, moisture, and temperature remain within tolerable ranges that the plant can effectively utilise. In other words, moderate canopy thinning facilitates beech regeneration without exposing the saplings to excessive ecological risk. Additionally, Anev (2024) reports

that following canopy removal, *F. sylvatica* responds physiologically by increasing the light compensation point, in its quest to adjust to increased light availability. These findings demonstrate that, despite its reputation as a shade-tolerant species, beech possesses considerable capacity for light acclimation under specific silvicultural or naturally disturbed conditions.

Populus tremula L. exhibits exceptionally high photosynthetic plasticity, allowing rapid adaptation to newly illuminated environments following canopy openings (Wittmann et al. 2001, Vodde et al. 2010). Such a strategy frequently results in the dominance of the species during the early successional stages following disturbance (Valladares and Niinemets 2008). *Acer platanoides* L. occupies an intermediate position between shade-tolerant and light-demanding species (San-Miguel-Ayanz et al. 2016). Although it favours partial shade, it demonstrates a moderate adaptive capacity to increased irradiance. This is achieved through both structural (Kloeppel and Abrams 1995) and functional leaf-level modifications, including variations in the content of photosynthetic pigments (Gitelson et al. 2009) and the photochemical activity of photosystem II (Lichtenthaler et al. 2013). These responses are consistent with the findings of Ciupak et al. (2019), who highlight the high seasonal plasticity of leaf physiology in this species.

The importance of light acclimation becomes even more pronounced in the context of climate change, where elevated temperatures, irregular precipitation, and the growing occurrence of extreme weather events increasingly constrain forest regeneration processes (Anderegg et al. 2020). Thus, the frequency of disturbances and the capacity of individual species to establish and persist in disturbed areas become critical to the future resilience of

forest ecosystems (Seidl et al. 2017). Investigating light acclimation in young individuals of *F. sylvatica*, *P. tremula*, and *A. platanoides* yields valuable insights into species-specific resilience and regeneration potential (Vodde et al. 2010). A detailed understanding of interspecific differences in light acclimation is essential for modelling forest dynamics and designing adaptive forest management strategies in the face of a changing climate (Brodribb et al. 2020).

The aim of this study was to assess interspecific differences in photosynthetic light acclimation and carbon-use efficiency in saplings of *Fagus sylvatica*, *Populus tremula*, and *Acer platanoides* following large-scale natural disturbances.

Materials and Methods

Objects and plant material

The gas exchange responses of European beech (*F. sylvatica* L.), Eurasian aspen (*P. tremula* L.), and Norway maple (*A. platanoides* L.) saplings to changes in light conditions following canopy disturbance were examined in the Petrohan training and experimental forest range, located in the Western Balkan Mountains. The selection of species was based on their contrasting ecological strategies: *F. sylvatica* as a shade-tolerant species adapted to low light availability; *P. tremula* as a pioneer, light-demanding species with high physiological plasticity; and *A. platanoides* as an intermediate strategist (San-Miguel-Ayanz et al. 2016). This design facilitated the assessment of interspecific differences in photosynthetic acclimation in response to disturbances of varying intensity and ecological impact.

The climate of the region is mountainous with moderate continental influence.

At an elevation of 1450 m, the mean annual temperature is 4.8 °C, while the average annual precipitation reaches approximately 1080 mm, with markedly higher values in spring (Kutchukova 1983, Koleva and Peneva 1990). According to Marinova and Bocheva (2023), the average temperature anomaly in Bulgaria for the reference period 1991–2020, compared to 1961–1990, was 0.8 °C. Unlike the average annual air temperature, no significant change in the annual precipitation amount was detected during this period. Marinova and Bocheva (2023) reported that temperature anomalies in the Western Balkan Mountains region ranged between 0.5 °C in autumn and 1.5 °C in summer. At the same time, precipitation rates have decreased by an average of 10–20 mm per year, particularly in winter and spring.

All three tree species were represented by naturally regenerated saplings growing in the same plots. The first sample plot (SP-14) was situated at an altitude of 876.1 m, 25.3° sloped in N-NE direction, and had been disturbed by windthrow in January 2014, ten years before measurements (Fig. 1).

The second sample plot (SP-21) was established in an area disturbed in 2021 by an ice storm, at an altitude of 946.8 m and with a 10.8° slope in the NE direction. A control plot (SP-0) was located between the two experimental plots. SP-0 lay within a closed-canopy forest, mono-dominated by *F. sylvatica*, approximately one-hundred-year old, at an altitude of 888.6 m with a 13.3° slope facing north, and had not been affected by disturbances in 2014 and 2021.

Gas-exchange measurements

Gas-exchange measurements were carried out under clear and cloudless skies on typical June days in 2024. For each

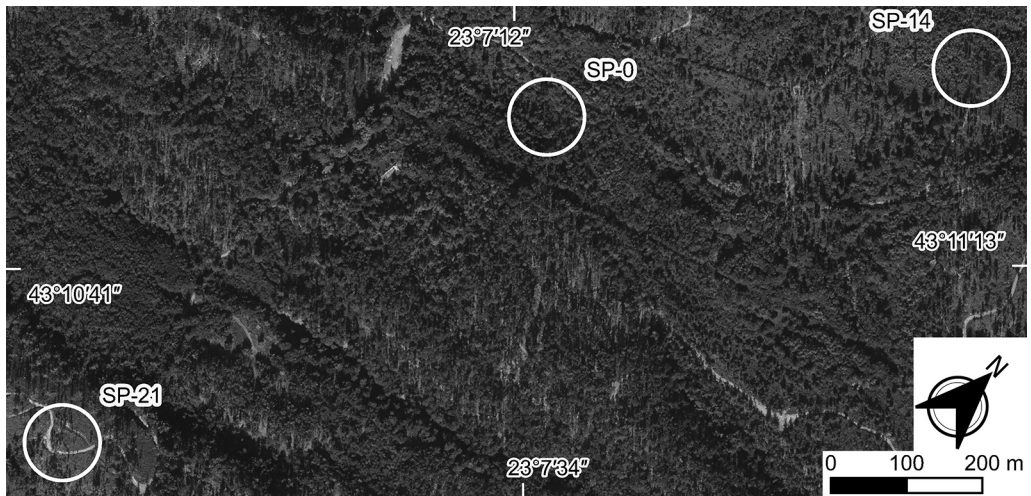


Fig. 1. Aerial view of studied region with sample plots (Inspire 2023).

measurement, five saplings per species (*F. sylvatica*, *P. tremula*, *A. platanoides*) were randomly selected on each plot. Net photosynthesis rate (A_N) was measured on the third fully expanded leaf, located on a branch from the middle section of the crown, using a Li-6800 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA) with Firmware v.2.1.11. The 3×3 cm leaf chamber (6800-12A; LI-COR) equipped with blue-red LEDs (6800-02; LI-COR) and a quantum PAR sensor (Li-190) was used. Prior to each measurement series, the instrument was calibrated according to the manufacturer's standard procedures (Li-Cor 2022). Each leaf underwent at least 3 min of acclimation at saturating irradiance ($1000 \mu\text{mol}(\gamma) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) until stable readings with a total coefficient of variation (TotalCV) below 0.5 % were achieved. The auto-programme 'Light Curve' was used to measure net photosynthesis at eight levels of photosynthetic photon flux density (PPFD), following a survey strategy (Li-Cor 2022). The 'Light curve' settings were light values: 2000, 1000, 500,

250, 100, 50, 25, 0; min wait: 60 s; max wait: 120 s; check early match. Before the measurements, the chamber environment was stabilised with a flow of $500 \mu\text{mol} \cdot \text{s}^{-1}$; fan speed 10,000 rpm; ambient and stable CO_2 (around 400 ppm), H_2O (around 50 %), and air temperature (around 25°C).

Photosynthetic light-response curves were analysed separately for each leaf by fitting a non-rectangular hyperbolic model (Thornley 1976, Prioul and Chartier 1977, Leverenz and Jarvis 1979, Marshall and Biscoe 1980, Norman et al. 1992). The following parameters were calculated: light-use efficiency ($\text{LUE}, \mu\text{mol}(\text{CO}_2) \cdot \mu\text{mol}(\gamma)^{-1}$), maximum photosynthetic rate ($A_{\text{MAX}}, \mu\text{mol}(\text{CO}_2) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), dark respiration rate ($R_D, \mu\text{mol}(\text{CO}_2) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and the convexity (θ) of the light-response curve. All regression curves were fitted using the least-squares method via the Newton algorithm in Microsoft Excel (Office 365, Microsoft, Redmond, WA, USA), using VBA script (Visual Basic for Application, v.7.1.1146, Microsoft, Redmond, WA, USA). The light compensation point ($\text{LCP}, \mu\text{mol}(\gamma) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)

was derived from the regression equations as the PPFD value, at which the rate of net photosynthesis equals zero ($A_N = 0$). One-way ANOVA tests and Fisher's LSD test were performed to assess the difference between the average values of A_{MAX} , R_D , LCP, LSP, LUE, and CUE of experimental sites. The carbon-use efficiency (CUE) was calculated as the ratio of the maximum photosynthetic rate (A_{MAX}) and the dark respiration rate (R_D), i.e. $CUE = A_{MAX} \cdot R_D^{-1}$. This approach reflects an approximation of the balance between carbon gain and respiratory carbon loss at the leaf level and has been adapted from the methodology proposed by Anev and Damyanova (2024) and Marinova and Anev (2023). It conceptually parallels the definition of CUE at the whole-plant and ecosystem scale, where CUE is commonly expressed as the ratio of net to gross primary production (NPP/GPP), as initially formulated by Thornley and Cannell (2000). Although alternative formulations exist – such as $CUE = (A_{MAX} - R_D) \cdot A_{MAX}^{-1}$ – these expressions are mathematically interrelated and provide equivalent biological interpretations within the physiological context of leaf gas exchange.

Results and Discussion

Analysis of the photosynthetic light-response curves indicated that the lowest rates of photosynthetic activity were consistently recorded beneath the forest canopy in all three investigated species (Fig. 2).

Following natural disturbances, *P. tremula* demonstrated rapid and pronounced acclimation. Saplings exhibited photosynthetic rates approximately 1.6 times higher than those observed beneath the canopy, achieving the highest A_{MAX} among the three species (Fig. 3).

This corresponds with the observations of Wittmann et al. (2001), who reported that *P. tremula* possesses high photosynthetic plasticity and shows rapid induction under fluctuating light conditions. Additionally, Roden and Pearcy (1993) noted that leaves of *Populus* species maintain high photosynthetic efficiency under variable light environments, highlighting their capacity for rapid adaptation to dynamic light regimes.

Nevertheless, the elevated rates of dark respiration (R_D), particularly in 2021, when they were twice those recorded be-

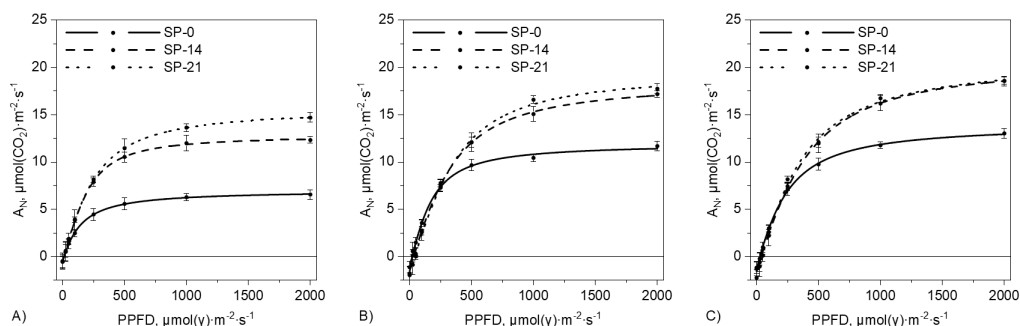


Fig. 2. Light curves of photosynthesis in A) *F. sylvatica*, B) *A. platanooides*, C) *P. tremula* saplings under the canopy (SP-0), in disturbed 2014 (SP-14), and disturbed 2021 (SP-21) sample plots.

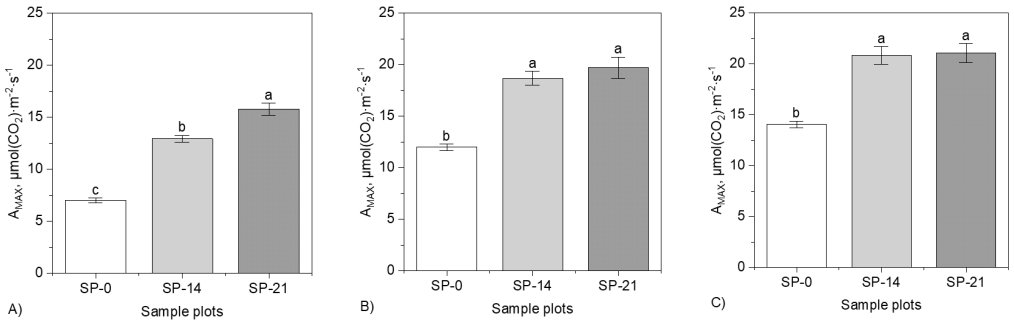


Fig. 3. Maximal photosynthesis in A) *F. sylvatica*, B) *A. platanoides*, C) *P. tremula* saplings under the canopy (SP-0), in disturbed 2014 (SP-14), and disturbed 2021 (SP-21) sample plots.

Note: The data are presented as mean $\pm$ SE. Means that do not share a letter are significantly different (One-way ANOVA with Fisher's LSD test, P -value < 0.05).

neath the canopy and 4.8 times greater than those recorded for *F. sylvatica*, indicate substantial carbon costs that may constrain the net carbon balance of the species (Fig. 4).

Furthermore, a study by Dai et al. (2021) has shown that elevated ozone levels can impair the thermal acclimation of photosynthesis in *P. tremula*, highlighting the species' vulnerability to combined stress factors, such as pollution and increased temperatures. *A. platanoides* also displayed substantial acclimation fol-

lowing disturbance, with increased values of A_{MAX} and LSP (figs 2 and 5).

Nevertheless, R_D in this species (Fig. 4) peaked in 2021, which may be attributed to elevated temperatures and drought conditions. A study by Carón et al. (2015) demonstrated that this species is highly sensitive to the combined effects of increased temperature and reduced soil moisture, resulting in diminished seedling survival and growth. These findings highlight the limited plasticity of *A. platanoides* under extreme climatic conditions.

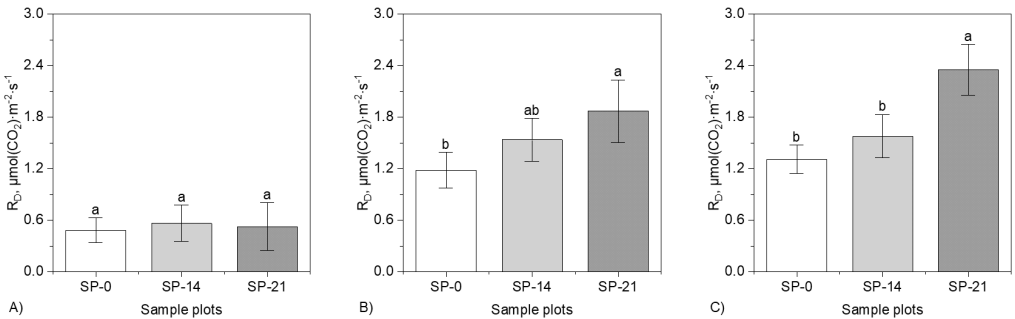


Fig. 4. Dark respiration in A) *F. sylvatica*, B) *A. platanoides*, C) *P. tremula* saplings under the canopy (SP-0), in disturbed 2014 (SP-14), and disturbed 2021 (SP-21) sample plots.

Note: The data are presented as mean $\pm$ SE. Means that do not share a letter are significantly different (One-way ANOVA with Fisher's LSD test, P -value < 0.05).

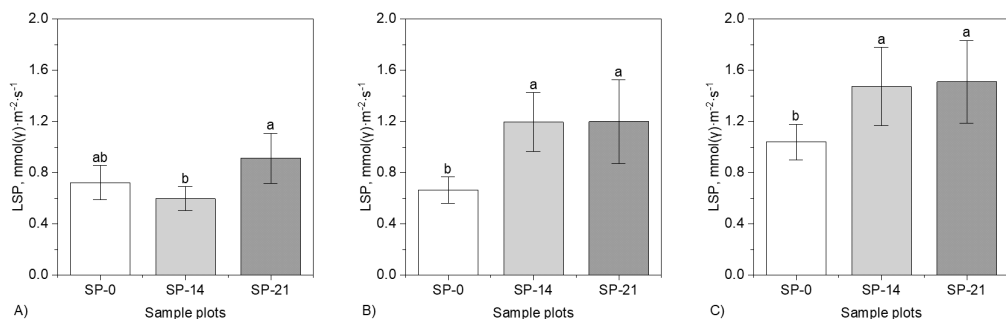


Fig. 5. Light-saturation points in A) *F. sylvatica*, B) *A. platanoides*, C) *P. tremula* saplings under the canopy (SP-0), in disturbed 2014 (SP-14), and disturbed 2021 (SP-21) sample plots.

Note: The data are presented as mean $\pm$ SE. Means that do not share a letter are significantly different (One-way ANOVA with Fisher's LSD test, P -value < 0.05).

A recent study by Urban et al. (2023) revealed that *A. platanoides* responds to drought by enhancing photoprotective mechanisms, specifically through an increase in non-photochemical energy dissipation (Φ_{NPQ}), which serves to mitigate the damaging effects of reactive oxygen species. However, even following the cessation of drought conditions, the species maintains elevated Φ_{NPQ} levels, suggesting a long-term physiological adaptation that may, nonetheless, result in reduced photosynthetic efficiency under chronic stress. These results confirm the species' moderate tolerance to water deficit and underscore the importance of carefully managing light and moisture regimes in regeneration contexts.

Moreover, research by Banks (2018) emphasises that *A. platanoides* exhibits distinct fluorescence characteristics under drought conditions, which may serve as indicators for assessing the physiological status of plants. This further supports the significance of *A. platanoides* as a competitively capable species under diverse environmental conditions, particularly in the context of ongoing climate change.

F. sylvatica, known for its shade tol-

erance, displayed a marked increase in photosynthetic activity in saplings exposed to open conditions following disturbance, reaching values up to 1.6 times higher than those recorded under dense forest shade. This enhancement was accompanied by low and stable rates of dark respiration (R_d), contributing to the highest CUE among the three studied species (Fig. 6).

Similar findings were reported by Kraj and Ślepaczuk (2022), who observed that under increased irradiance, beech leaves adapt through structural modifications and increased leaf area, while maintaining photosynthetic efficiency and antioxidant defence. These results underscore the ability of *F. sylvatica* to successfully adjust to varying light regimes without compromising physiological stability. Anev and Marinova (2021) further demonstrated that, despite an initial decline in photosynthetic activity following windthrow events, saplings of this species recovered water-use efficiency and chlorophyll content within few years, indicating long-term physiological adaptation to altered light conditions.

Morphological plasticity, including var-

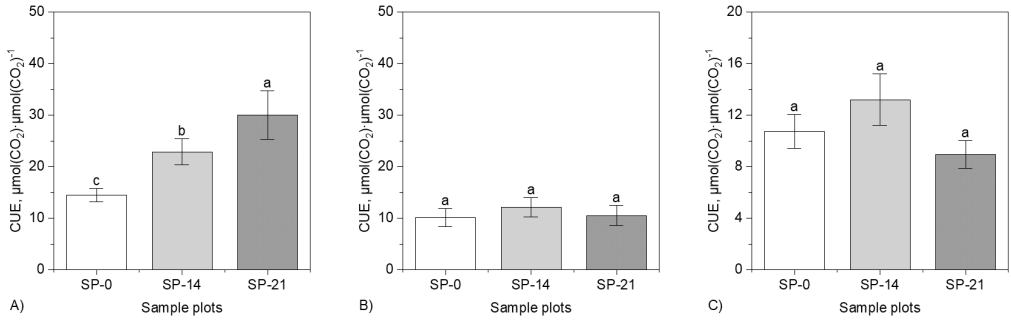


Fig. 6. Carbon-use efficiency in A) *F. sylvatica*, B) *A. platanooides*, C) *P. tremula* saplings under the canopy (SP-0), in disturbed 2014 (SP-14), and disturbed 2021 (SP-21) sample plots.

Note: The data are presented as mean $\pm$ SE. Means that do not share a letter are significantly different (One-way ANOVA with Fisher's LSD test, P -value < 0.05).

iation in leaf area, also contributes to the acclimation potential of beech saplings under changing environmental conditions. Anev et al. (2016) demonstrated that leaf area in *F. sylvatica* can be reliably estimated using a non-destructive model based on linear dimensions, which reflects underlying structural adjustments to light availability. These adaptations, when combined with physiological responses, support the species' capacity to maintain carbon balance and optimise light capture in heterogeneous environments.

This capacity for resilience is not limited to abiotic factors. *F. sylvatica* has also exhibited biochemical defence responses under biotic stress. Lamom et al. (2016) reported that herbivory by *Orchestes fagi* induced significant oxidative stress in beech leaves, particularly at lower altitudes, as reflected in elevated malondialdehyde (MDA) and proline levels. These findings highlight the species' ability to initiate antioxidative mechanisms while sustaining physiological performance. In addition, Anev and Damyanova (2024) found that *F. sylvatica* maintains stable light- and carbon-use efficiency throughout the growing season, even under seasonal fluctuations

in light intensity, confirming the species high plasticity.

Recently, a study by Provazník et al. (2024) investigated the photosynthetic performance of *F. sylvatica* saplings originating from different altitudes and geographical regions and planted in Denmark. The results indicated that saplings from higher elevations exhibited superior photosynthetic efficiency and adaptation to the new conditions, underscoring the importance of provenance in determining the species' adaptive potential. These findings reinforce the status of *F. sylvatica* as a species with high physiological resilience and adaptability to changing environmental conditions.

A comparative analysis of light compensation points (LCP), *F. sylvatica* had the lowest values, consistent with its shade-tolerant nature and capacity for efficient photosynthesis under low light availability. In contrast, *P. tremula* and *A. platanooides* showed higher LCP values, particularly in saplings examined in 2014 and 2021, suggesting greater light requirements to sustain a positive carbon balance (figs 7 and 8).

These findings are supported by re-

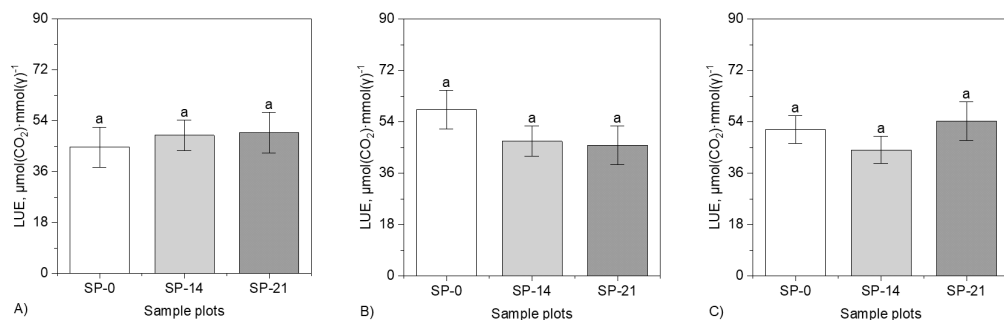


Fig. 7. Light use efficiency of photosynthesis in A) *F. sylvatica*, B) *A. platanoides*, C) *P. tremula* saplings under the canopy (SP-0), in disturbed 2014 (SP-14), and disturbed 2021 (SP-21) sample plots.

Note: The data are presented as mean $\pm$ SE. Means that do not share a letter are significantly different (One-way ANOVA with Fisher's LSD test, P -value < 0.05).

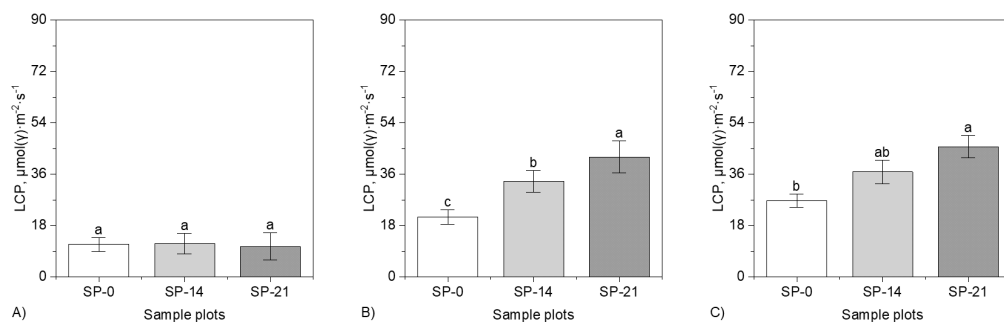


Fig. 8. Light compensation points in A) *F. sylvatica*, B) *A. platanoides*, C) *P. tremula* saplings under the canopy (SP-0), in disturbed 2014 (SP-14), and disturbed 2021 (SP-21) sample plots.

Note: The data are presented as mean $\pm$ SE. Means that do not share a letter are significantly different (One-way ANOVA with Fisher's LSD test, P -value < 0.05).

search conducted by Pfanz et al. (2002), which demonstrated that *F. sylvatica* possesses exceptionally low LCP and high light-use efficiency, rendering it highly competitive in shaded habitats. Conversely, a study by Kloeppel and Abrams (1995) reported that *A. platanoides* exhibits higher LCP values and lower photosynthetic efficiency under low light conditions, thereby limiting its capacity for growth in shaded environments.

In summary, the results clearly illustrate substantial interspecific differences

in both the extent and nature of light acclimation and carbon-use efficiency among saplings of *P. tremula*, *A. platanoides*, and *F. sylvatica* following natural disturbances to the forest canopy. *P. tremula* exhibits exceptionally rapid photosynthetic adaptation to altered conditions; however, this is accompanied by elevated dark respiration rates, which may potentially offset the benefits of accelerated carbon flux. *A. platanoides* displays a moderate photosynthetic response and a degree of resilience, yet remains sensitive to wa-

ter deficit and thermal stress, which may compromise its long-term competitive potential. In contrast, *F. sylvatica*, traditionally classified as a shade-tolerant species, demonstrates high physiological stability, characterised by low respiratory losses and high carbon-use efficiency, underscoring its capacity to maintain productivity under limited light availability. These species-specific differences are crucial for understanding their respective roles in forest regeneration processes and for predicting the structural dynamics of mixed forests in the context of increasing ecological disturbances.

Conclusions

The present study emphasises the pivotal role of photosynthetic plasticity in the acclimation of tree species to changing environmental conditions. The findings demonstrate that *P. tremula* and *A. platanoides* exhibit a high degree of light acclimation, as evidenced by increased values of maximum photosynthesis (A_{MAX}) and light saturation point (LSP) following natural disturbances. Nevertheless, *F. sylvatica* displayed the highest CUE, attributed to its low dark respiration rates, which underscores its physiological resilience and adaptability to shaded environments. Natural disturbances substantially affect the photosynthetic activity of young trees, leading to marked alterations in key physiological parameters, such as A_{MAX} , LSP, R_d , and LCP. These changes are strongly species-specific and highlight the need for a differentiated approach to evaluating regeneration potential and promoting sustainable forest development under changing climatic conditions. Understanding interspecific differences in adaptation mechanisms is essential for optimising sil-

vicultural strategies, including regeneration management, habitat restoration, and the conservation of forest ecosystems. In this context, the integration of physiological indicators into models of future forest development may enhance the effectiveness of adaptive management in the face of increasing ecological uncertainty.

Acknowledgements

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Colorimetric analysis of eggshell colour variations in farm-raised Common pheasant (*Phasianus colchicus* Linnaeus, 1758)

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Abstract

The aim of the study was to perform a colorimetric assessment of eggshell colour variations in Common Pheasant (*Phasianus colchicus* Linnaeus, 1758) used for resettlement in Bulgaria. A total of 360 eggs were divided into five groups according to eggshell colour. Using a colorimeter, the colour characteristics were determined in the CIE L*a*b* colour space, and chroma (C) and the Shell Color Index (SCI**) were calculated. The highest lightness (L*) values were observed in the beige eggshells (68.62 ± 1.62), while the lowest were found in the olive-green eggshells (55.89 ± 0.49) ($P < 0.001$). Low negative mean values for the a* coordinate were observed only in the green eggs (-1.41 ± 0.78), while low positive values were recorded for the other groups, reaching 6.8 ± 0.93 for olive-green eggshells. Positive values were recorded for the b* coordinate, ranging from 13.96 ± 0.80 (green) to 21.90 ± 0.77 (beige), indicating a yellow spectrum position ($P < 0.001$). Shell Color Index (SCI**) had the lowest values for olive-green eggshells (-35.07 ± 1.42) and the highest values for green eggshells (-53.07 ± 2.17) ($P < 0.01$). The study revealed significant variations in the pigment distribution in the eggshells of the Common Pheasant, with differences in the colour index ranging up to 51.67 % between the maximum and minimum individual values. It is possible that these differences in eggshell colouration are linked to the genetic purity of the most widely bred subspecies of the Common Pheasant (*Phasianus colchicus mongolicus* Brandt, 1844) in the country's game farms. Conducting a phylogenetic analysis of the population could shed light on this issue.

Key words: chroma, egg, lightness, Shell Color Index.

Introduction

Common pheasant (*Phasianus colchicus* Linnaeus, 1758) is the most widely distributed species within the Phasianidae family, with a natural range covering much of temperate Asia and possibly extending westward into southeastern Europe (Madge and McGowan 2002). As a valuable game bird, it has been introduced to various parts of the world, primarily Europe and North America, but also to Chile, Japan, Australia, New Zealand, and Tasmania (Fuller and Garson 2000, Madge and McGowan 2002, BirdLife International 2016, Avery 2019). Its extensive range is associated with notable intraspecific variation, resulting in a wide subspecific diversity (Kayvanfar et al. 2017). In this context, based on certain phenotypic differences – primarily in males – approximately 30 subspecies of the Common pheasant are recognised, which can be grouped into five main groups (Madge and McGowan 2002, Braasch et al. 2011). A more recent study based on phylogenetic analyses of various subspecies identifies eight distinct evolutionary lineages, suggesting that the species could be taxonomically reclassified into three distinct species (Liu et al. 2020). A major threat to the genetic integrity of individual subspecies is hybridisation with introduced birds released for hunting purposes (Ashrafzadeh et al. 2021, Aliakbari et al. 2021, Boev 2025). Due to its widespread distribution and the routine release of captive-bred birds into the wild, the species is classified as 'Least Concern' by the IUCN, with a global population estimated between 160 and 220 million mature individuals, less than 5 % of which are found in Europe (BirdLife International 2016). Among European countries, the United Kingdom hosts the largest share, accounting for approximately

45 % of the population (Avery 2019).

Ring-necked pheasants, commonly referred to as 'game' or 'hunting pheasants' in some countries, are often hybrids of various subspecies selected for desirable hunting traits and represent the most economically important form of the species in Europe and North America (Sova et al. 1974, Fuller and Garson 2000, Korzh 2001, Shurulinkov et al. 2005, Péteľis 2011). This is also the most hunted landfowl species globally, accounting for approximately 40 % of all landfowl harvested (Quaresma et al. 2016). In the United Kingdom alone, between 44 and 47 million birds are released annually, with about one-third subsequently harvested (Aebischer 2019). The species shows significant potential for production of high-quality, game-type meat (Marsico and Vonghia 1991). In several countries, mainly in Europe and the USA, large populations of Common pheasants are also bred for meat production, in addition to release into the wild (McCready and Mitchell 1969, Adamski and Kuźniacka 2006, Kokoszyński et al. 2024). For this purpose, white meat-type pheasant lines have been developed (Roberson 2006).

The native subspecies in Bulgaria, *Phasianus colchicus colchicus* Linnaeus (1758), became extinct in the wild approximately 50 years ago as a result of long-term releases of introduced subspecies and subsequent hybridisation (Boev 2025). Three main subspecies of Common pheasant have been introduced into the country: *Phasianus colchicus torquatus* Gmelin (1789), *Phasianus colchicus mongolicus* Brandt (1844), and *Phasianus colchicus karpowi* Buturlin (1904) (Gruychev 2014). In Bulgaria, two major farms rear pheasants with exterior traits similar to *P. c. colchicus* for release purposes, while most other breeding facilities

focus on *P. c. mongolicus*. Annually, approximately 20,000 black-necked pheasants are released in Southern Bulgaria (Gruychev 2014), and around 90,000 ring-necked pheasants – mainly resembling *P. c. mongolicus* – are released in the rest of the country (EFA 2020). On average, just over 20% of the released pheasants are harvested annually in Bulgaria (Mihaylov et al. 2014).

The significant role of Common pheasant in game management across many regions of the world, as well as its value as a model species for studying the effects of climate change and human activity on ecosystems, has been the focus of numerous scientific studies (Stoate 2002, Musil and Connelly 2009, Chiatante and Meriggi 2022, Krüger 2022, Asgharzadeh et al. 2023). Increasing research attention has also been directed towards growth performance and meat quality, due to the species' notable dietary and nutritional properties (Roberson 2006, Adamski and Kuźniacka 2006, Kotowicz et al. 2012, Quaresma et al. 2016, Kokoszyński et al. 2024, Wagner et al. 2025). An interesting aspect is that, although there are numerous studies on the morphological characteristics of Common pheasant eggs (Kirikçi et al. 2003, Kirikçi et al. 2005, Kożuszek et al. 2009, Krystianiak et al. 2016a, Galic et al. 2023), detailed data on eggshell colour based on modern colorimetric analysis are lacking. Some studies have focused on the relationship between eggshell colour in Common pheasants and egg quality (Richards and Deeming 2001, Krystianiak et al. 2005, Kożuszek et al. 2009, Krystianiak et al. 2016b) or incubation traits (Hulet et al. 1978, Ristić et al. 2013, Nowaczewski et al. 2018, Biesek et al. 2024), generally categorising eggs into several main colour groups: blue, olive, dark brown, and light brown.

Studies indicate that pheasant eggshell colour is associated with egg quality and incubation traits. Eggs with weakly expressed pigmentation often show shell defects, particularly in microscopic structure and thickness, which may contribute to their lower hatchability (Krystianiak et al. 2016a, 2016b), highlight potential biological costs of pigmentation. In this context, the aim of the present study was to perform a colorimetric assessment of eggshell colour variations in Common pheasant (*Phasianus colchicus*) used for resettlement in Bulgaria.

Materials and Methods

Experimental design

For the purposes of this study, eggs from Common pheasants (*Phasianus colchicus*), bred in captivity at 'Dunav' pheasantry in Ruse, Northern Bulgaria, and used for resettlement, were collected. Based on origin and exterior characteristics, the birds are attributed to the subspecies *Phasianus colchicus mongolicus*. The parental flock from which the eggs were obtained was 10 months old. A total of 360 eggs were classified into five groups according to eggshell colour: green (various forms of biliverdin deposition; $n = 134$), grey-green (various forms of predominant biliverdin deposition; $n = 51$), beige (various forms of protoporphyrin deposition; $n = 12$), beige-green (various forms of predominant protoporphyrin deposition; $n = 117$), and olive green (intense deposition of protoporphyrin combined with biliverdin; $n = 46$) (Fig. 1). The eggs were fresh (randomly collected on a single day: 24th February 2024) and stored at +16 °C until the following day, when colorimetric analysis was performed after tempering for



Fig. 1. Representative eggs from the five main colour groups based on eggshell pigmentation in the Common pheasant (*P. colchicus*): green (1), grey-green (2), beige (3), beige-green (4), and olive green (5).

three hours at room temperature (+23 °C) in the Egg Quality Laboratory of the Poultry Science Unit, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

Data collection

A portable colorimeter PCE-CSM 2 (PCE Instruments SE) was used for the colorimetric analysis. The device is equipped with a Silicium photodiode sensor, an LED light source (illuminant D65), and a measuring aperture of $\varnothing$ 8 mm. Measurements were carried out in the CIE $L^*a^*b^*$ colour space. Each egg was measured three times – at the equatorial zone, the pointed pole, and the blunt pole. Based on these measurements, mean values for L^* , a^* , and b^* were calculated for the three zones of each egg. To better visualise the results, the eggshell colour data for each egg were further summarised by averaging the values obtained from the three measurement points by formulae (1), (2), and (3).

$$L^* = (av L^*p + av L^*e + av L^*b) \cdot 3^{-1}, \quad (1)$$

$$a^* = (av a^*p + av a^*e + av a^*b) \cdot 3^{-1}, \quad (2)$$

$$b^* = (av b^*p + av b^*e + av b^*b) \cdot 3^{-1}, \quad (3)$$

where: $av L^*p$, $av a^*p$, and $av b^*p$ represent the average values of the three axes (L^* , a^* , and b^*) at the pointed pole; $av L^*e$, $av a^*e$, and $av b^*e$ are the average values at the equatorial zone; and $av L^*b$, $av a^*b$, and $av b^*b$ correspond to the average values at the blunt pole.

Based on the measurements performed in the CIE $L^*a^*b^*$ colour space, chroma (C^*) and the Shell Color Index (SCI^{**}/MCI) were calculated, applying a correction coefficient (-1) for eggs with visible biliverdin pigmentation (green, grey-green, beige-green, and olive green), according to the formulae (4), (5), and (6).

$$C^* = \sqrt{a^{*2} + b^{*2}} \text{ (CIE 1976)}, \quad (4)$$

$$SCI^{**} = (L^* - C^*) \text{ (Lukanov et al. 2018)}, \quad (5)$$

$$SCI^{**} = (L^* - C^*) \cdot (-1) \text{ (Lukanov et al. 2016, 2018)}. \quad (6)$$

Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics (v.26) with one-way ANOVA. The following descriptive parameters were used: mean ($\bar{x}$), standard deviation (SD), and standard error of the mean (SEM). Results are presented as mean $\pm$ SEM. Intergroup differences were considered statistically significant at $P < 0.05$, based on the LSD *post hoc* test, provided the data were normally distributed and the ANOVA model was significant ($P < 0.05$, F-test). Microsoft Excel 16.0 (2018, Windows) was used for graphical visualisation.

Results and Discussion

The grey-green and beige-green groups accounted for the largest proportions of the sample (37.22 % and 32.5 %, respectively), whereas the beige group was least represented (3.33 %) (Table 1). Because the present study employed a colour-grouping methodology that differs from those in previous reports, direct comparison with published data on the most prevalent eggshell colour in the species is not possible. Under the current pigment-based classification – defined by biliverdin and protoporphyrin deposition

– approximately two-thirds of the eggs showed biliverdin deposition with moderate to high protoporphyrin levels, while the remaining one-third exhibited either low or very intense protoporphyrin deposition, corresponding to the green and olive-green groups. The primary pigments responsible for avian eggshell colouration are biliverdin (biliverdin IX α and its zinc chelates) and protoporphyrin (protoporphyrin-IX). In addition, other pigments such as bilirubin, uroerythrin, and coproporphyrin-III have also been identified in certain bird species (Liu and Cheng 2010, Hamchand et al. 2020, Stoddard 2022, Wang et al. 2023a). In the present study, we focus on the two primary pigments associated with avian eggshell colouration, while not excluding the possibility of trace amounts of other pigments being present.

L* represents the lightness of a colour, ranging from black (0) to white (100). In the case of eggshells, low L* values correspond to dark colours, while high values indicate lighter colours (Aygun 2013). Eggs with unpigmented (white) shells exhibit the highest L* values, whereas those with intense protoporphyrin or biliverdin deposition (dark brown or dark green) show the lowest (Lukanov et al. 2015a, Genchev et al. 2022a). The Common pheasant eggs studied demonstrated rel-

Table 1. CIE L*a*b* colorimetric characteristics of egg colour groups of Common pheasant ($\bar{x} \pm$ SEM).

Parameter	Eggshell colour group				
	green n=134	grey-green n=51	beige n=12	beige-green n=117	olive green n=46
L	67.56 $\pm$ 1.88a	61.31 $\pm$ 0.77b	68.62 $\pm$ 1.62c	63.65 $\pm$ 1.10d	55.89 $\pm$ 0.49abcd
a	-1.41 $\pm$ 0.78abc	3.35 $\pm$ 0.61	6.97 $\pm$ 0.21a	4.19 $\pm$ 0.62b	6.80 $\pm$ 0.93c
b	13.96 $\pm$ 0.80abc	18.26 $\pm$ 0.62d	20.77 $\pm$ 0.77ad	18.50 $\pm$ 0.71b	19.63 $\pm$ 1.15c
C	14.48 $\pm$ 0.66abcd	18.67 $\pm$ 0.98ae	21.90 $\pm$ 0.81be	19.02 $\pm$ 0.77c	20.82 $\pm$ 1.30d

Note: With the same letters (a-a, b-b) are marked statistically significant between-group differences for each sex at $P < 0.01$.

atively similar mean L^* values, except for the olive-green group, ranging from 68.62 ± 1.62 in the beige group to 61.31 ± 0.77 in the grey-green group ($P > 0.05$). Significantly lower mean L^* values were observed in eggs with the most intense protoporphyrin and biliverdin pigmentation – olive green (55.89 ± 0.49 , $P < 0.01$). The highest individual L^* value was recorded in green eggs (79.54), and the lowest in olive green eggs (54.1). The greatest within-group variation in lightness was found in the green and beige eggs, while the olive-green group showed the least variation. In contrast to Common pheasant eggs, chicken eggs with biliverdin pigmentation exhibit even greater variation in L^* , likely related to the degree of pigmentation and the combination of the two main pigments, biliverdin and protoporphyrin, across different chicken breeds (Lukanov et al. 2015a, 2015b; Lukanov et al. 2016; Drabik et al. 2021; Wang et al. 2023b). It has been established that eggshell pigmentation in laying hens varies in intensity depending on the production period, gradually becoming lighter over time due to the dynamic change in the content of protoporphyrin IX (Odabaşı et al. 2007, Bi et al. 2018). A similar trend is observed in the eggshell colour of Common pheasant, where pigmentation is genetically determined, but its intensity tends to fade as the laying period progresses (Kožuszek et al. 2009).

The a^* axis measures the colour balance between green and red, with positive a^* values indicating a reddish spectrum and negative values corresponding to a greenish tone (CIE 1976). In the present study, low positive mean a^* values were found for almost all colour groups of Common pheasant eggs, except for the green group. Although the eggs visually exhibit different shades of green, except for the

beige group, only the green group showed a slight influence of biliverdin. This may be explained by a stronger effect of protoporphyrin on the colorimetric analysis, which masks the green pigmentation. Nevertheless, differences in mean a^* values among the groups were small, ranging from 6.97 ± 0.21 in the beige group to -1.41 ± 0.78 in the green group ($P < 0.01$). Individual minimum and maximum values varied from -9.14 in the green group to 8.95 in the olive-green group. In contrast to Common pheasant eggs with visible green colouration (groups green, grey-green, beige-green, and olive green), chicken eggs with biliverdin pigmentation generally show predominantly negative a^* values, including those with combined biliverdin and protoporphyrin pigmentation (Lukanov et al. 2015a, 2015b; Li et al. 2021; Wang et al. 2023b, 2023c; Xu et al. 2024). This indicates an inverse relationship in chicken eggs compared to Common pheasant eggs, reflecting a stronger influence of the green pigment on a^* values in chickens.

In contrast to a^* , the b^* axis showed exclusively positive, moderately high mean values for all tested egg groups. It measures the colour deviation between blue and yellow, with positive values associated with the yellow spectrum and negative values with blue, placing all Common pheasant eggs within the yellow spectrum. All egg groups exhibited relatively similar mean values, with a more pronounced distinction observed in the green eggshell group (13.96 ± 0.80) (Table 1). Individual measurements varied more widely, ranging from 6.63 (green) to 23.46 (olive green). Compared to Common pheasant eggs, chicken eggs with biliverdin pigmentation generally display lower, but still positive or comparable b^* values (Lukanov et al. 2015b, Drabik et al. 2021, Wang et al. 2023a, Beranova et

al. 2024, Xu et al. 2024). In Greater Rhea (*Rhea americana* Linnaeus, 1758), the eggshell colour is yellow, corresponding to higher b^* values (30.28 ± 0.72) compared to Common pheasant eggs, which colour is visibly within the green spectrum (Genchev et al. 2022b).

In the CIE Lab* colour space, chroma (C^*) represents colour saturation or intensity – indicating how vivid or dull it appears. Low C^* values correspond to desaturated or dull colours, while high C^* values indicate vivid, intense, and saturated colours (CIE 1976). Analysis of C^* across different colour groups of Common pheasant eggs shows that the mean values correspond closely to those of b^* . This relationship is directly linked to the way chroma is calculated and the relative influence of the a^* and b^* axes. In our case, b^* has a considerably stronger impact, whereas a^* , with mean values close to zero, exerts a weaker influence on C^* . Individual C^* values for Common pheasant eggs ranged from 10.91 (green) to 24.03 (olive green). The lightest green

eggs in the study exhibited more intense colouration compared to eggs with solely biliverdin pigmentation found in certain chicken breeds or hybrids (Li et al. 2006; Lukanov et al. 2015a, 2015b).

The results for the modified shell colour index (SCI^{**}) in the CIE Lab* colour space, initially proposed by Lukanov et al. (2018) for meat colour determination and subsequently applied as a more objective measure for eggshell colour assessment (Genchev et al. 2022a), are presented in Figure 2.

Due to the presence of visibly green pigmentation, a correction factor (-1) was applied to differentiate the index values of eggs with greenish shells from those with brown pigmentation. Consequently, eggs from the green, grey-green, beige-green, and olive-green groups exhibit negative SCI^{**} values (Lukanov 2016). Eggs in the beige group, lacking visible green pigmentation, were not subjected to this correction and thus show positive SCI^{**} values. The lowest mean SCI^{**} value was observed in the olive-green group (-35.07

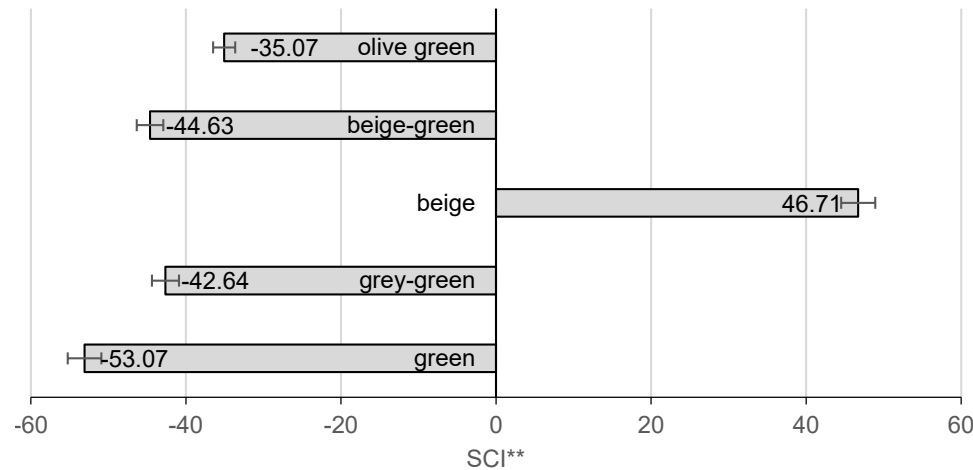


Fig. 2. Mean Summary Colorimetric Index (SCI^{**}) values of egg colour groups of Common pheasant.

± 1.42), while the highest was recorded for the beige group (46.71 ± 2.2) ($P < 0.001$). Among the eggs with visible green pigmentation, the green group exhibited the highest SCI^{**} values (-53.07 ± 2.17), which correlates with its high L^* and low a^* and b^* values (Table 1). Application of the shell colour index produces comparable (Lukanov et al. 2015a; Lukanov et al. 2016) or slightly higher (Li et al. 2006, Drabik et al. 2021, Xu et al. 2024) results for biliverdin-pigmented eggs of Common pheasants and domestic chickens, after recalculating SCI^{**} from published data. For comparison, emu eggs, which also exhibit biliverdin pigmentation, show significantly lower SCI^{**} values (Genchev et al. 2022a).

Conclusions

Based on our classification method, approximately two-thirds of Common pheasant eggs exhibit biliverdin deposition with moderate to high levels of protoporphyrin, while the remaining one-third show either minimal or very intense protoporphyrin deposition, highlighting the predominance of grey-green and beige-green colour groups. The analysed Common pheasant eggs exhibited relatively consistent mean L^* values across colour groups, with the lowest lightness observed in olive-green eggs – characterised by the most intense biliverdin and protoporphyrin pigmentation - while intra-group variation was greatest in green and beige eggs. The generally weak positive a^* values observed across most Common pheasant egg colour groups, aside from the green group, suggest a dominant influence of protoporphyrin over biliverdin in determining eggshell colour, potentially masking the green pigmentation in the colorimet-

ric analysis. All pheasant eggs exhibited positive b^* values, indicating yellow-spectrum eggshell colouration with limited intergroup differences, though individual measurements showed notable variability. Application of the corrected SCI^{**} index allowed clear separation between green- and brown-pigmented pheasant eggshells, with the most negative values recorded for the green group – reflecting high L^* and low a^* and b^* components – and the highest positive values for beige eggs lacking visible green pigmentation.

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The carob moth, *Apomyelois ceratoniae* (Zeller, 1839) (Lepidoptera: Pyralidae): a newly reported pest of common walnut in Bulgaria

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Thematic field: Agriculture in mountainous and semi-mountainous regions

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Abstract

The common walnut (*Juglans regia* L.) is widely distributed in Bulgaria, commonly cultivated in private gardens, along streets and intercity roads, and in orchards. It currently ranks third among fruit tree species in terms of cultivated area in the country. Walnut is also important in agroforestry systems, including alley cropping, silvopastoral, and forest farming. This study aimed at the early detection of alien insect species in Bulgaria. Field surveys were carried out in August and September 2024 in urban and suburban localities of southern Bulgaria where common walnut trees are established. Larvae and pupae of Carob moth *Apomyelois ceratoniae* (Zeller, 1839) were found in dry walnut fruits collected in areas of Belovo, Chernichevo, Chernomorets, Gorski izvor, Kapitan Andreevo, Lyubimets, Plovdiv, Purvomay, Simeonovgrad, and Svilengrad. This is the first report of *A. ceratoniae* as a pest of common walnut in Bulgaria. The species appears to be widely distributed and may be more prevalent than currently documented.

Key words: agroforestry, alien, distribution, insecta, *Juglans regia*.

Introduction

The carob moth *Apomyelois ceratoniae* (Zeller, 1839) originates from the Mediterranean region and is now widely distributed across six continents due to its accidental introduction as a contaminant

of agricultural products (Heinrich 1956, Abedi et al. 2019). In Europe, the species has been reported in Albania, Austria, Belgium, Croatia, Cyprus, the Czech Republic, Denmark, France, Georgia, Germany, Greece, Hungary, Italy, the Netherlands, North Macedonia, Norway, Poland, Por-

tugal, Romania, Russia, Slovenia, Spain, Sweden, Switzerland, Türkiye, and the United Kingdom (Karsholt and Razowski 1996, Lopez-Vaamonde et al. 2010, Morland 2015, Demirel 2016, Plant and Jakšić 2018, CABI 2025, GBIF 2025, Observation.org 2025).

The species was first recorded in Bulgaria by Roesler (1969). In 2005, the carob moth was detected near Kozhuh, close to Petrich (Plant 2016). More recently, the species was isolated from seed pods of *Gleditsia triacanthos* L., collected in September and December 2022 in the Upper Thracian Plain, near the village of Gelemenovo (Gradinarov et al. 2024). The carob moth is a highly polyphagous insect, having been recorded on 43 host species from 18 plant families, including 21 plants of significant economic value (Wakil et al. 2015). It was initially recognised as a Mediterranean pest of stored fruits and nuts (Zeller 1839, Morland 2015). Numerous studies have reported its larvae feeding on seeds and pods of a wide range of plants, including *Punica granatum* L., *Citrus* spp., *Pistacia vera* L., *Juglans regia* L., *Prunus dulcis* (Mill.) D.A. Webb, *Macadamia integrifolia* Maiden & Betche, *Acacia farnesiana* (L.) Willd., *Caesalpinia sappan* L., *Cassia bicapsularis* L., *Ricinus* spp., *Erythrina monosperma* Lam., *Haematoxylum campechianum* L., *Prosopis juliflora* (Sw.) DC., *Samanea saman* (Jacq.) Merr., *Phoenix dactylifera* L., and *Ceratonia siliqua* L. (Plant 2016). The primary cultivated hosts of economic concern include carob, citrus, medlar, almond, fig, pomegranate and date palm (Doumandji 1981, Demirel 2016, Dueñas-López 2022).

During a survey aimed at the early detection of alien insect species in Bulgaria, larvae and pupae of an unknown moth species were found inside fruits of the common walnut (*Juglans regia*) in south-

ern Bulgaria.

The common walnut is widely distributed in Bulgaria, commonly grown in private yards, along streets and intercity roads, and as a cultivated fruit tree. Currently, walnut ranks third among fruit tree species in terms of cultivated area in Bulgaria (MAF 2024). Walnut also has broad applications in agroforestry and can be integrated into alley cropping, silvopastoral, and forest farming systems (Stancheva et al. 2015).

This communication reports *A. ceratoniae* as a newly observed pest of common walnut in Bulgaria.

Materials and Methods

Field surveys were conducted from August to September 2024 in urban and suburban areas with walnut trees (*J. regia*) located in southern Bulgaria. Visual inspections of walnut trees were carried out, and fruits showing signs of infestation were collected. The fruits were dissected, and some of the larvae found inside were preserved for further identification. A portion of the larvae was reared to adulthood on their naturally infested hosts in laboratory conditions.

Only fruits containing larvae were recorded as infested by the carob moth. Identification was performed on adult specimens using the morphological description provided by Morland (2015) and mothdissection.co.uk, and on larvae according to Solis (1986). Altitudinal data for each locality where the moth was recorded are included. The distribution of the species is mapped using the UTM grid system, based on the template provided by Abadjiev (2001). The material has been deposited in the author's collection at the University of Forestry, Sofia.

Results

Material examined: Chernomorets, 30 m a.s.l., 02.08.2024: 1 fruit 1 larva; Belovo, 310 m a.s.l., 12.08.2024: 1 fruit with 1 larva; Chernichevo, 274 m a.s.l., 20.09.2024: 1 fruit with 1 larva; Gorski izvor, 163 m a.s.l., 20.09.2024: 2 fruits, each with 1 larva; Kapitan Andreevo, 50 m a.s.l., 20.09.2024: 1 fruit with 1 larva, 1 fruit with 2 larvae and 1 pupal exuvium; Lyubimets, 56 m a.s.l., 20.09.2024: 1 fruit with 1 larva; Plovdiv, 160 m a.s.l., 20.09.2024: 2 fruits, each with 1 larva; Purvomay, 13 m a.s.l., 20.09.2024: 1 fruit with 1 pupa; Simeonovgrad, 100 m a.s.l., 20.09.2024: 1 fruit

with 5 larvae; Svilengrad, 55 m a.s.l., 20.09.2024: 1 fruit with 5 larvae, 1 fruit with 2 pupal exuviae, 1 pupa and 2 larvae.

Larvae found in common walnut fruits (*J. regia*) and adults obtained from reared preimaginal stages were identified as the carob moth (*A. ceratoniae*). It is a medium-sized moth with a characteristic forewing colour pattern (Fig. 1). The larvae (Fig. 2) and pupae (Fig. 3) were detected in fruits with a dry pericarp. All larvae were observed feeding on the kernel. Pupation occurs inside the fruit (Fig. 3), and after eclosion, the pupal exuvium protrudes from the fruit (Fig. 4). An adult emerged from a larva collected on 02.08.2024 in



Fig. 1. *A. ceratoniae*, adult specimen.



Fig. 2. *A. ceratoniae*, larvae.



Fig. 3. *A. ceratoniae*, pupa.



Fig. 4. *A. ceratoniae*, pupal exuvium protruding from the fruit upon eclosion.

Chernomorets on 18.08.2024. Larvae collected in September overwintered under laboratory conditions, and adults emerged between March and April. Between 1 and 5 larvae per fruit were recorded. *A. ceratoniae* was detected in 10 localities in urban and suburban areas of southern Bulgaria, at altitudes up to 310 m a.s.l. (Belovo) (Fig. 5).

Discussion

Although *A. ceratoniae* is best known as a pest of pomegranate, fig, and pistachio, several peer-reviewed studies have documented its ability to infest walnut as a host plant (Catling 1979; Solis 1986; Gonzalez and Cepeda 1999; Mozaffarian et

al. 2007a, 2007b). In the late 1990s, the carob moth was identified as a significant pest of walnuts in Chile (Gonzalez and Cepeda 1999).

Despite the existence of these studies, there is still a lack of detailed research on the bioecology of populations developing specifically on walnut. The available data mostly pertain to the species' development on other host plants.

A. ceratoniae overwinters in all larval stages within fruits that have fallen to the ground or remained unharvested from the previous season (Gianessi 2009). The number of generations per year and the developmental success of the species depend on several factors, including temperature, humidity, photoperiod, host species, and host quality (Gugliuzzo et

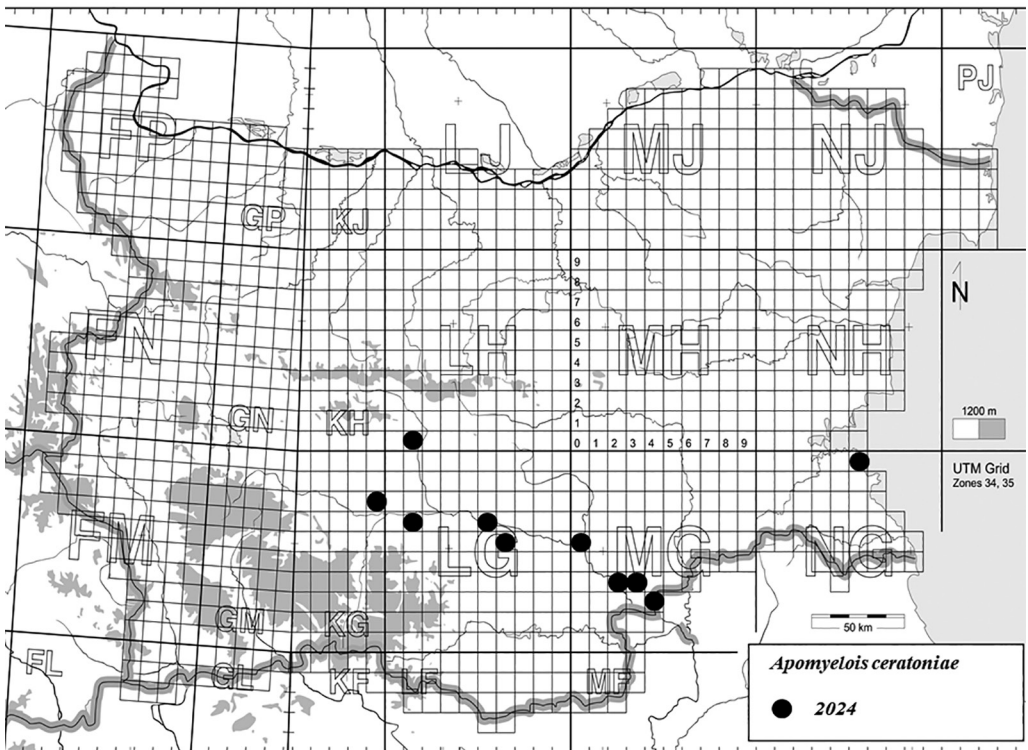


Fig. 5. Distribution of *A. ceratoniae* in Bulgaria (2024), based on UTM grid.

al. 2019). According to Mozaffarian et al. (2007a), it emerges in late April in Iran and lays eggs on any available suitable host. Its phytophagous larval stage can damage leaves, shoots, and fruits of various host plants (Klem and Zaspel 2019).

Different authors report varying numbers of generations depending on the host plant. For example, Blumberg (2008) states that *A. ceratoniae* can complete up to four generations annually under the agro-ecological conditions of Mediterranean date palm orchards. Yıldırım and Başpınar (2015) found the species present from May to November in pomegranate orchards, with adult populations peaking in August. Moreover, Demirel 2016 reported that the seasonal activity of carob moth increased from June to October, while it declined from April to May and October to November. The percentage of the total moths caught over this period was highest in October and September.

In Bulgaria, *A. ceratoniae* was previously recorded in the pods of *Gleditsia triacanthos* L. collected in September and December 2022 (Gradinarov et al. 2024). We detected the species on walnut during August–September 2024, suggesting that earlier generations may have developed on alternative host plants. Our findings are consistent with observations in Iran, where specimens were collected from fig, pistachio, and walnut in September and November, while moths emerging in early May primarily infested pomegranate and were collected from this host in July and August (Mozaffarian et al. 2007b).

Mozaffarian et al. (2007a) reported low levels of infestation by *A. ceratoniae* on hosts other than pomegranate. However, larvae developing in pistachio, fig, and walnut produced larger moths than those reared on pomegranate, indicating successful internal feeding. The authors con-

cluded that the expansion of host range in the second half of the season may support better overwintering and larger population sizes. They further suggested that fig, pistachio, and walnut may contain nutritional factors that promote the growth of late-season larvae and adults, and may play a more significant role in overwintering success than previously infested pomegranates.

Although the species is widespread in Europe, Liu et al. (2024) predicted that under current climate conditions, the most suitable habitats for *A. ceratoniae* are in southwestern Asia, northern Africa, and southern Europe (including Portugal, France, Italy, and Spain).

The role of *A. ceratoniae* as an orchard pest has been studied primarily in the Mediterranean region (Gugliuzzo et al. 2019, Yıldırım and Başpınar 2015, Longo and Tirrò 2005), in the Western Cape of South Africa (Morland 2015), and in Iran (Mozaffarian et al. 2007a, 2007b). However, it remains unclear whether the species acts as a primary pest on walnut or colonizes fruit already damaged by other pests or pathogens. To date, there are no reports of *A. ceratoniae* occurring on walnut in Europe.

Unlike many other orchard pests, the carob moth can reproduce during storage (Navarro et al. 1986) and is considered a detritivorous species that feeds on stored products such as dried fruits, dates, nuts, carob, and pistachios (Lopez-Vaamonde et al. 2010). It is a major pest of stored almonds in Israel (Calderon et al. 1969).

Conclusion

The primary host crops attacked by *A. ceratoniae* – carob, citrus, medlar, fig, pomegranate, and date palm – have lim-

ited economic significance in Bulgaria. Although the species has not yet been reported as a storage pest in the country (Tomov and Trencheva 2013), it poses a potential risk to stored dried fruits and nuts.

Although only isolated specimens of *A. ceratoniae* were found in the present study, further research is needed to assess its distribution and host range in Bulgaria, as well as its potential impact as a pest of walnut – particularly in light of the growing interest in walnut cultivation across the country.

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Productivity and essential oil content of thyme varieties, cultivated in the Eastern Rhodopes, Bulgaria

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Thematic field: Agriculture in mountainous and semi-mountainous regions

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Abstract

Thyme is a perennial herb from Lamiaceae family, particularly valued for its essential oils, which contain bioactive compounds with significant therapeutic and culinary properties. The oil composition varies significantly due to factors like genetic diversity and geographical distribution, resulting in distinct chemotypes. Thyme cultivation offers a valuable alternative to harvesting natural resources which disturb and diminish biodiversity and natural sustainability. A comparative field study was set up in 2021 at the Agricultural Experimental Station at the Eastern Rhodopes in town of Kardzhali with thyme species. Three genotypes were included in the study: a thymol-type cultivar 'Deutscher Winter' (*Thymus vulgaris* L.), and two Bulgarian cultivars: citral-type 'Slava' (*Thymus sibthorpii* Benth.) and geraniol-type 'Pagane' (*Thymus marschallianus* Willd.). Phenological and biometrical indexes were gathered and analysed, data on oil content and mass productivity were also presented. In the Eastern Rhodopes full bloom period is cultivar-dependent and occurs as follows: 10–20 May for 'Deutscher Winter' thyme, 1–10 June for 'Slava' and 5–20 June for 'Pagane'. At the third year of thyme cultivation, the highest fresh mass per plant was gathered from 'Deutscher Winter' variant (445 g), followed by 'Slava' (345 g) and 'Pagane' (219 g). The highest FW/DW ratio was evaluated for 'Slava' (1:4). In the first years, fresh mass yield harvested per hectare varied significantly: 10–13 t·ha⁻¹ from 'Deutscher Winter', 6–13 t·ha⁻¹ from 'Slava' and 2–7 t·ha⁻¹ from 'Pagane'. Essential oil harvest quantities followed fresh mass yield – the highest oil yield was recorded for 'Deutscher Winter' (19.6–24.5 kg·ha⁻¹), followed by 'Slava' (12.8–32.8 kg·ha⁻¹) and 'Pagane' (8.1–17.3 kg·ha⁻¹). Oil content was dependent on the year of cultivation. The highest amount was recorded for 'Pagane', followed by 'Slava' and 'Deutscher Winter'.

Key words: bioproductivity, thyme essential oil, *Thymus marschallianus*, *Thymus sibthorpii*, *Thymus vulgaris*.

Introduction

Thyme is a perennial herb from Lamiaceae family, particularly valued for its essential oil, which contain bioactive compounds with significant therapeutic and culinary properties. Thyme essential oil is rich in volatile components such as thymol, geraniol, citral, etc., which contribute to its antibacterial, antifungal and antioxidant properties (Mancini et al. 2015). The oil composition varies significantly due to factors like genetic diversity and geographical distribution, resulting in distinct chemotypes (Niculae et al. 2019, Kryvtsova et al. 2022).

Bulgaria is among the European countries with very high *Thymus* species diversity – over 18 species (Aneva et al. 2018). The richest floristic region – the Rhodopes mountains, contain 80 % of them. In fact, the Rhodopes contain largest number of species of Bulgarian medicinal flora in general – 148 species, which represent 80 % of total medicinal flora of the country (Tashev and Tsavkov 2008). This concentration is most likely connected to the phytogeographic origin of the species, thus defining Rhodopes as a region with favourable agroclimatic conditions for *Thymus* distribution. Many species are thriving in natural habitats with high conservation values (Biserkov 2015). Harvesting natural resources disturb and diminish biodiversity and natural sustainability, thus thyme cultivation would offer a valuable alternative. For elaboration of proper cultivation techniques and field schemes, however, biological and morphological characteristics of thyme cultivars need to be registered.

The genus *Thymus* is characterised with vast genetic diversity which determines difficulties in their phytochemical identification (essential oil composition).

For the purposes of the pharmaceutical industry mostly, harvested herba from different species of certain region are labelled *Thymus* sp. and manufactured further. However, the medicinal activity and culinary value of *Thymus* species largely depends on their essential oil composition which differ enormously depending on the species. The content of the predominant monoterpenes such as geraniol, thymol, citral, etc., in the oil determines its chemotype. In general, chemotype determination is done based on the main essential oil compounds with a relative percentage of over 10 %. In exceptional cases, further significant compounds of slightly lower GC% (over 8 %) were also indicated.

The research done on characterisation of chemical composition and antibacterial activity of thyme species is extensive (Čavar Zeljkovic and Maksimovic 2015, Dos Santos e Silva et al. 2022, Etri and Pluhár 2024). In addition, thyme oil content and composition is strongly affected by environmental factor and agronomical practises (Badi et al. 2004, Najar et al. 2021).

Research on breeding and cultivation on thyme species in Bulgaria started in 1980s. Preliminary selection process yielded three genotypes with distinct phenotypic features. Subsequent oil analyses revealed also distinct chemical composition of essential oils distilled from their herbs. Thus, three cultivars were produced: a geraniol-type thyme 'Pagane', a citral-type thyme 'Slava' and a carvacrol-type genotype 'Kresna' (Stoeva et al. 2001).

The cultivar 'Pagane' is a mutation from *Thymus marschallianus* Willd. population, introduced in Bulgaria from Czech Republic (Yankulov and Yankulova 2008). *T. marschallianus* is a Eurasian species, in Europe mostly distributed in northern

parts. The variety is not part of the Bulgarian medicinal flora. The cultivar is sterile and propagates by cuttings or tuft division. It forms large creeping tufts with multiple half-upright grassy stems. The stems, inflorescence and flowers are thin and gentle, with limited wooden base. Flowers are dark purple. The essential oil composition of 'Pagane' is dominated by geraniol and closely resembles the aroma of so-called Hungarian thyme.

Initially, 'Slava' was reported to be selected from citral-type population of *Thymus vulgaris* L. (Yankulov 2007). Subsequently, botanical and chemical features of the genotype mostly confirmed resemblance to *Thymus sibthorpii* Benth. variety. The plant forms low loose shrub, with half-wooded stalks and light purple flowers. The predominant component in its essential oil is also geraniol, but high amounts of citral isomers were also detected, which contributes to its lemony or citrus odour, particularly in fresh mass (Zhekova 2013). Dry herbal commercial product of this species is known as 'kekik' and widely used in the Turkish cuisine (Alan and Koca 2007).

The cultivar 'Deutscher Winter' was introduced from Germany and originates from *Thymus vulgaris* with thymol chemotype. It is vastly distributed in Europe and mostly grown for culinary purposes. In contrast to previous two cultivars, the plant forms compact upright sub-shrubs, detached from the soil surface. The habitus is vigorous, with strong shoots, mostly wooden in base. The predominant compound in its essential oil is thymol and its precursors – *p*-cymene and α -terpinene (Zhekova et al. 2011).

The aim of the present study was to evaluate the growth and productivity of three thyme genotypes with distinct oil chemotype when cultivated in open field

in the Eastern Rhodopes in Bulgaria.

Material and Methods

Experimental area

A comparative open field study was set up in 2021 at the Agricultural Experimental Station in the Eastern Rhodopes near town of Kardzhali (41°35'28.7" N and 25°23'49.8" E). The region belongs to Southern Bulgarian Xerothermic soil zone, within shallow cinnamonic forest soil sub-zone of Eastern Rhodopes region. Typical for the region are the cinnamon forest soils. The humus layer is 15–40 cm, with dense and hard structure (Sapundzhiev and Mitreva 2016). The upper soil layer is very low in humus (2 %), with low nitrogen and phosphorus content and high potassium content. The soil physical qualities are unfavourable because of its low water permeability.

The Eastern Rhodope region is low-mountainous and hilly. The climate is typically southern – in winter precipitation is mostly rain. Dry summers and warm winters are typical for the region. Annual precipitation is 700–800 mm, the average annual air temperature is 12–13 °C (Sapundzhiev and Mitreva 2016).

Cultivar choice and productivity assessment indexes

Three thyme cultivars were included in the on-field evaluation:

1. 'Deutscher Winter' (*Thymus vulgaris* L.) – a thymol-type cultivar;
2. 'Slava' (*Thymus sibthorpii* Benth.) – citral-type cultivar;
3. 'Pagane' (*Thymus marshallianus* Willd.) – geraniol-type cultivar.

For determination of growth and pro-

ductivity of thyme variants biometric measurements were conducted annually on 10 plants (tufts) of each variant (30 plants per variant) at the beginning of full bloom and include following indices:

- plant height, cm – measuring in the tufts centre from soil surface up to the tip of the inflorescences mass;
- plant diameter, cm – measurement from end to end of tuft towards rows;
- fresh weight of plant mass, g – measured immediately after harvest;
- dry weight of plant mass, g – measured after air drying in shade for 14 days.

A one-way ANOVA was applied to show significant differences among *Thymus* cultivars concerning their growth and development.

Essential oil distillation

For determination of oil content aerial parts of all variants were harvested in full bloom, weighted and left to dry in shade for at least two weeks. Oil content was determined in air-dried parts (flowers and leaves) after steam distillation in a five-li-

tre flasks with distillation time 75 min using a Clevenger type apparatus.

For climate analyses were used primary climatic data gathered from the Synoptic station – Kardzhali of the National Institute of Meteorology and Hydrology (NIMH). Anomaly calculation was done using the new standard climatic norm for 1991–2020 period.

Results and Discussion

Effect of meteorological factors of thyme cultivars phenology

Thyme’s growth and development dynamics depends on the meteorological factors with two periods being of greatest importance. The first is last decade of March to mid-April, which determines spring growth, normal stem formation and bush enlargement. The seconds is mid-May – mid-June, when flowering occurs. As seen from Table 1, during the trial period 2022–2024, the primary meteorological factors (air temperature and precipitation)

Table 1. Climate data for 2022–2024 vegetation period.

Years	Mean monthly air temperature, °C						AVG, °C	Anomaly, °C
	III	IV	V	VI	VII	VIII		
2022	3.9	11.7	16.2	21.1	24.3	23.7	16.82	- 0.01
2023	8.2	9.9	15.0	19.9	22.9	22.3	16.37	-0.46
2024	9.0	14.5	15.1	23.9	25.3	24.1	18.65	+1.82
norm (1991–2020)	8.7	11.2	16.0	20.0	22.6	22.5	16.83	-

Years	Monthly sum of precipitation, mm						Sum, °C	Anomaly, %
	III	IV	V	VI	VII	VIII		
2022	50.8	97.2	62.0	54.6	26.2	63.4	354.2	104
2023	52.4	134.1	67.8	94.3	19.1	27.1	394.8	116
2024	95.3	59.7	94.7	19.7	33.4	34.4	337.2	99
norm (1991–2020)	75.1	57.3	66.1	66.2	46.9	27.7	339.3	-

Note: Anomaly calculation was done using the new standard climatic norm for 1991–2020. Source: Synoptic station Kardzhali, NIMH.

differed both from each other and from the climatic norm.

In 2022 and 2023 average air temperature and precipitation were almost equal to climatic norms with no significant anomalies. 2024 was the warmest and driest, with early start of vegetation caused by higher spring temperatures and low water amounts. In addition, an anomaly of over 1.5 °C was recorded for the overall vegetation period as well as for each month. In that year growth of all cultivars started 10 days earlier than in the others, flowering period was shorter and due to summer drought did not form second vegetation cycle at all.

Thyme cultivars differ in their flowering periods due to their different origin (Table 2). 'Deutscher Winter' is an early-flowering cultivar, its full bloom is ceased till end of May the latest. Flowers of 'Pagane' and 'Slava' develop later, hence flowering ends till middle of June. Similar results regarding the flowering of 'Pagane' and 'Slava' were reported by Yankulov and Yankulova (2008) and Yankulov (2007). Blooming time depends on air temperature in March and April and in 2024 it started earlier for all cultivars. The probable reasons were higher spring temperatures and lack of moisture registered for that year.

Analyses of biometrical and productive characteristics over three-years period

The trial period (2022–2024) confines second to fourth year after establishing

thyme plantation or first to third harvesting years. The data revealed that thyme cultivars differ substantially in their biological characteristics and productivity. The results and conclusions are presented in Figure 1 and tables 3 and 4.

Dynamics of thyme tufts growth at first years after planting in field reveals that the tufts of all variants gradually enlarge despite that they are being harvested every year (inflorescences were harvested). It also indicates that all cultivars exerted a good overwinter survival, which means that the environment is appropriate for them. Up to the third year tuft width reached 70 cm, height varied depending on the cultivar and was measured from 14 to 25 cm. 'Deutscher Winter' and 'Slava' tend to be more vigorous than 'Pagane'. 'Deutscher Winter' established a large upright shrub already on the second year, with no significant variation in height every year – from 25 to 29 cm. Other authors (Mewes et al. 2008) report on 19–25 cm height of 'Deutscher Winter' plants – slightly lower numbers, perhaps due to environmental conditions. The growth of 'Slava' and 'Pagane' was slower and plants enlarged constantly for the studied period. Thus, on third harvesting year their growth was not ceased and shrubs enlargement could be expected further. On third year both variants do not exceed 25 cm height.

Thyme plants enlargement is followed by increase in their productivity which was higher each following year for every variant (Table 3). The highest fresh mass increase was recorded for 'Deutscher

Table 2. Flowering periods of thyme cultivars across different years.

Year	'Deutscher Winter'	'Slava'	'Pagane'
2022	15 – 20 May	25 May – 10 June	25 May – 15 June
2023	20 May – 10 June	1 – 10 June	5 – 20 June
2024	10 May – 1 June	1 – 10 June	1 – 10 June

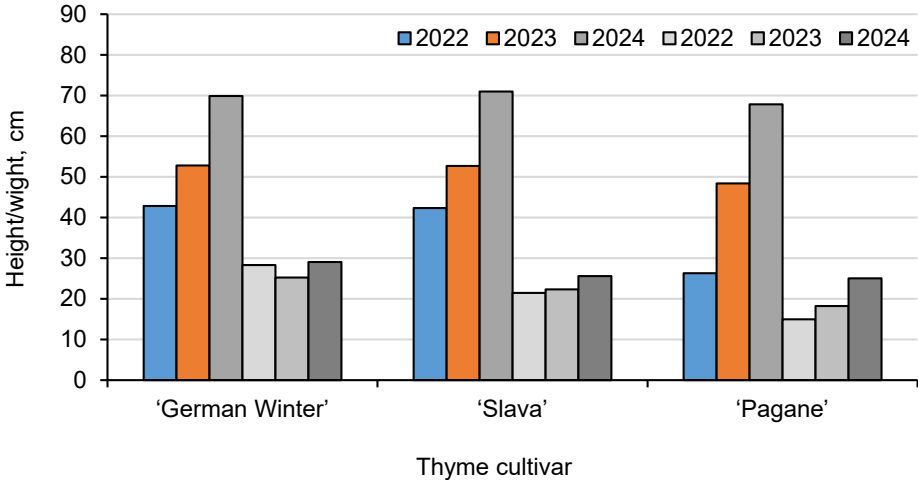


Fig. 1. Growth dynamics of thyme cultivars in the first years after planting.
Note: coloured bars represent tuft width (or diameter), grey bars – tuft height.

Table 3. Productivity indices per plant of thyme cultivars for 2022–2024 period.

Cultivar	Fresh weight, g	Dry weight, g	FW/DW	Oil content, w%
2022				
'Deutscher Winter'	247.97c	64.30c	3.9	0.19
'Slava'	176.17b	40.74b	4.3	0.22
'Pagane'	75.00a	24.70a	3.0	0.39
2023				
'Deutscher Winter'	368.33c	105.57b	3.5	0.23
'Slava'	322.77c	90.33b	3.6	0.28
'Pagane'	167.03a	48.40a	3.5	0.31
2024				
'Deutscher Winter'	445.60b	154.53a	2.9	0.18
'Slava'	344.67c	112.20b	3.1	0.24
'Pagane'	219.17a	67.87c	3.2	0.19

Note: Mean values, followed by different letters are statistically different at $p = 0.05$.

Winter' variant (248–446 g per plant) – almost doubled for three years. Similar trend was registered for the plants of 'Slava' (176–345 g per plant) despite the lower quantities. The fresh mass yield of 'Pagane' was the lowest – below 100 g per plant in the first year – but increased significantly by the third year, exceeding

200 g per plant. The most important index for spice production is FW/DW ratio because it determines dry spice yield that will remain after fresh mass is desiccated. On average for all cultivars the ratio ranged between 1:3 and 1:4. 'Deutscher Winter' ratio varied between 2.9 and 3.9 – almost the same

results were reported by Król and Kieltyka-Dadasiewicz (2015) for that cultivar. Lower values were recorded for 'Pagane', because it forms thinner grassy stems that contain more water. FW/DW ratio depends predominantly on the years' conditions, mostly on precipitation amounts in spring. Still, that conclusion needs confirmation in subsequent years since plants are in still enlarging.

Essential oil content differed among cultivars significantly and was not dependent on years of cultivation. The richest in essential oils were herb from 'Pagane' – up to 0.33 w%. Opposite to fresh mass trend, oil quantities distilled from 'Deutscher Winter' were the lowest – average for the study period 0.2 w%. Higher amounts were recorded for 'Slava' – 0.25 w%. Other author (Yankulov 2007) reported 0.3 % to 0.6 % essential oil content for 'Slava' in third harvest year. Higher values were also reported by the same author for the oil content of the 'Pagane' cultivar, ranging from 0.55 % to 0.75 %.

Dynamics of fresh mass and essential oil yield

Fresh mass yield of all variants has been increasing up to third year of plantation in field. Here the calculation was made based on planting scheme of the experimental plot, which contains 47,610 plants per hectare. Productivity indexes follow thymes growth trends – vigorous variants produced higher yields earlier. 'Deutscher Winter' thyme fresh mass yield was above 10 t·ha⁻¹ already on the second year, reaching almost 13.5 t·ha⁻¹ on the third year. The yield of 'Slava' was low in the second year after planting, but in the third year it reached the levels of 'Deutscher Winter' – 13.6 t·ha⁻¹. Fresh mass of 'Slava' variant was reported by Yankulov

(2007) to be 16 t·ha⁻¹ on the second to 13 t·ha⁻¹ on the third year after planting in field. 'Pagane' variant produced lower amounts fresh mass – above 7 t·ha⁻¹ in 2024. This result is similar to that published by other authors (Yankulov and Yankulova 2008), who reported average yield of fresh mass of 8.3 t·ha⁻¹. Here, average calculations on thyme fresh mass and essential oil yield were avoided intentionally, because the thyme plantation was still young when data recording initiated. Still, data published from other authors concerning herbage yield is very diverse, because of the diverse genotypes and ecological conditions of thyme growing (Król and Kieltyka-Dadasiewicz 2015). Nevertheless, they all stress the importance of precipitation amount for yield formation (Table 4).

Table 4. Yield of fresh mass and essential oil from thyme cultivars for 2022–2024.

Year	Fresh mass yield, t·ha ⁻¹	Essential oil yield, kg·ha ⁻¹
'Deutscher Winter'		
2022	10.29	19.6
2023	10.25	23.6
2024	13.62	24.5
'Slava'		
2022	5.80	12.8
2023	12.54	35.1
2024	13.66	32.8
'Pagane'		
2022	2.07	8.1
2023	5.58	17.3
2024	7.36	14.0

Essential oil yield of different cultivars, however, differed among years and was not connected to inflorescence yield rise. 'Deutscher Winter' produced 19–24.5 kg·ha⁻¹ oil, higher amounts were recorded for 'Slava' variant – 12–33 kg·ha⁻¹. From 'Pagane' herb was distilled the lowest quantities thyme oil – 8–17 kg·ha⁻¹.

Conclusions

Comparative study on growth and productivity of thyme cultivars 'Deutscher Winter' (*Thymus vulgaris*), 'Slava' (*Thymus sibthorpii*) and 'Pagane' (*Thymus marshallianus*) in their first years after planting in open field revealed significant differences among their phenology and productivity. Some characteristics as growth dynamics and fresh mass productivity are genotype-dependent – 'Deutscher Winter' is early flowering, vigorous cultivar, that reaches its potential already on the second year. In comparison, on their third year 'Slava' and 'Pagane' still enlarge, thus implying that the growth potential has not been reached yet. FW/DW ratio and oil content, most likely depend on year's environmental conditions, since their values differ among the years for each thyme variant. To confirm these trends more accurately, data should be collected in following years. At the current state of the experiment, on the third year of thyme plantation can be concluded that plants enlargement is not ceased and further yield rise could be expected. Distinct phenological and biometric differences among the cultivars were observed, which sets some data for elaboration of cultural practise and optimal plant density schemes.

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Operational assessment of the FAE SSM/HP 250 forestry tiller for soil preparation in the establishment of field protection forest belts

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Thematic field: Forest operations and wood processing

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Abstract

This paper presents results of an experimental study of a FAE SSM/HP 250 forestry tiller, powered by Fendt 939 Vario tractor with nominal power of 265 kW, used for soil preparation of open areas designated for field-protection establishment of forest belts in the Dobrudzha region in Northeastern Bulgaria. The influence of the mechanical composition and hardness of the soil, the tillage depth, and the tiller rotor rotational frequency on the operational performance and fuel consumption were studied. The experiments were conducted in open forest areas, with different mechanical composition and hardness of the soil. The experimental plots are located on clay-sandy soils with an average hardness of 345 kPa, sandy-clay soils with hardness 690 kPa and clay soils with hardness 2100 kPa. As a result of the study, functional mathematical models were developed to determine the operating performance and fuel consumption of the tilling unit, depending on the rotor rotational frequency, soil mechanical composition and hardness, and tillage depth. These results were used to determine the optimal rotor speed of the tiller based on soil type and tillage depth.

Key words: fuel consumption, mechanical composition, operational performance, rotor rotation frequency, soil hardness, tilling depth.

Introduction

Over the past 10–12 years, the use of reclamation and specialised forest tillers for soil preparation for afforestation has begun in the forestry holdings of the

Northwest State Enterprise 'Vratsa' and the Northeast State Enterprise 'Shumen'. From the research conducted so far in our country and abroad on the use of this type of machinery and technology, a high quality of soil preparation has been estab-

lished, as well as the reduction of some operations and the time for afforestation (Gönnä and Marc 1992; Hallbrook et al. 2006; Löf et al. 2012; Marinov 2014; Keča and Pajić 2015; Marinov and Jordanova 2017; Kostov 2019a, 2019b; Marinov and Kostov 2019).

Along with the positive aspects, some weaknesses were also identified, such as higher energy costs, more expensive maintenance, etc. (Marinov 2017, 2019a; Kostov 2019a, 2019b; Furmanov et al. 2020; Glushkov et al. 2021). Forest milling machines in our country have been studied mainly in the preparation of areas for poplar plantations establishment along the Danube, Iskar and Ogosta rivers (Jordanova and Marinov 2015, Marinov 2019b, Marinov and Peev 2024).

The research carried out so far on the milling machines for preparing the areas for the field protection forest belts establishment in our country is mainly focused on the machines for shredding wood waste and bushes, stumps and roots (Marinov and Kostov 2022, Marinov et

al. 2023). In order to establish the operational properties of the forest tillers for soil preparation for the field protection forest belts establishment, an experimental study was carried out with the FAE SSM/HP 250 forestry tiller in the forest territories of the North-Eastern State Enterprise.

Aim of the research was to establish the way of the combined influence of rotation frequency of working organs of forest tillers, tillage depth, mechanical composition and hardness of the soil, on the operational performance and fuel consumption of the tiller units, in soil preparation of open forest areas.

Object of Study

The object of the study was the FAE SSM/HP 250 forestry tiller, powered by a Fendt 939 Vario tractor, with a rated power of 265 kW (Fig. 1).

Subject of study was the operational performance of forest tillage machines, namely: hourly performance; hourly and



Fig. 1. FAE SSM/HP250 forestry tiller powered by a FENDT 939 Vario tractor (photo: K. Kostov).

relative fuel consumption, optimal frequency and speed of rotation of working elements.

The study was conducted in open areas designated for the establishment of field protection forest belts in the area of the state forestries in the towns of Dobrich, Balchik and Tervel, from 'Shumen' North-Eastern Enterprise.

Material and Methods

The experimental studies were conducted in open forest areas, on flat and slightly sloping terrain, and slightly rocky soils, with different mechanical composition. The experimental areas were located on clay-sandy soils with hardness $H = 345$ kPa, sandy-clay soils with hardness $H = 690$ kPa and clay soils with hardness $H = 2100$ kPa. The stoniness, hardness and humidity of the soil, as main factors of the working environment, are maintained at certain levels during the experiments. In all experimental areas, the soil is slightly stony.

Soil hardness is measured with a portable Wile cone penetrometer at separate control points that start at the beginning of the experimental plots and move in a diagonal direction towards the end of the plot. For this purpose, between 40 and 50 stitches are made on an area of 0.1 ha, or a minimum of one stitch on an area of 25 m². The maximum penetration depth of the device is 40 cm, and finally the average soil hardness in each test area is determined. Soil moisture is measured with a portable Walcom RMS-710 soil moisture meter, with a probe length of 28 cm. Soil moisture was determined at the same points where soil hardness was measured with the cone penetrometer. During the experimental observations, the

soil moisture in the assigned experimental areas was within 22–29 %, which favours its tilling. Fuel consumption per hour is measured and reported by the tractor's on-board computer. An electronic stopwatch is used to timing the operations. Garmin-550T GPS is used to measure the areas. To measure linear dimensions, a Bosch GLM 80 laser tape measure with a range of up to 80 m and an accuracy of ± 0.1 mm, and a Yucon Extend LRS-1000 laser range-finder with a range of up to 1000 m and an accuracy of ± 1 m.

Experimental design

To achieve the objective of the experimental study, the influence of three main factors on the operational performance of forestry tillers was examined, namely: rotor rotation frequency of the tiller, depth of the tilling, and mechanical composition and hardness of the soil. The first factor was managed and maintained at nine levels, the second and third factors at three levels.

Input independent parameters (x_i)

The input manageable and controllable factors and their variation levels are determined by the operating conditions and technological parameters of the machine. In the present study, these factors are:

- x_1 – rotor rotation frequency of the tiller with 9 levels of variation: $n = 840 \text{ min}^{-1}$, 860 min^{-1} , 880 min^{-1} , 900 min^{-1} , 920 min^{-1} , 940 min^{-1} , 960 min^{-1} , 980 min^{-1} , and 1000 min^{-1} ;
- x_2 – tillage depth with 3 level of variation: $a = 20 \text{ cm}$, 30 cm , and 40 cm ;
- x_3 – soil hardness with 3 levels of variation: $H = 345 \text{ kPa}$, 690 kPa , and 2100 kPa .

The input unmanageable factors,

which are kept constant at one level during the experiment, are: rated power of the tilling unit – $N_{\text{nom}} = 265$ kW, working width – $B_p = 2.56$ m, average slope of the terrain – $2-3^\circ$, relative soil humidity – $W_a = 22-29\%$.

To facilitate the computational procedures, the natural values of the three input factors were transformed into dimensionless (coded) quantities, since they have different dimensions. For this purpose, the middle of the variation interval of the real (natural) factors is determined. The coding of the factors is carried out according to equation (1) (Vuchkov and Stoyanov 1986).

$$\dot{x}_i = \frac{x_i - x_{i,0}}{\lambda_i} \quad (1)$$

where: $\dot{x}_i$ is coded value of the i th factor; x_i – the natural value of the i th factor; $x_{i,0}$ – the natural value of the middle of the i th factor; λ_i – interval of variation of the i th factor.

The coded values of the levels of variation of the input factors are: (-1) for the lower level, (0) for the initial level and (+1) for the upper level. The coded values of the three factors are:

- The initial (basic) level of the first controllable factor x_1 – rotor speed is $x_{1,0}$

$= (840 + 1000) \cdot 2^{-1} = 920 \text{ min}^{-1}$. The variation interval is $\lambda_1 = 1000 - 920 = 80 \text{ min}^{-1}$, and the coded values of the first factor are $x_i = (x_1 - x_{1,0}) \cdot \lambda_1^{-1}$;

- The initial (basic) level of the second controllable factor x_2 – soil tillage depth is $x_{2,0} = (20 + 40) \cdot 2^{-1} = 30 \text{ cm}$; The variation interval is $\lambda_2 = 40 - 30 = 10 \text{ cm}$, and the coded values of the second factor are $x_i = (x_2 - x_{2,0}) \cdot \lambda_2^{-1}$;

- The initial (basic) level of the third controllable factor x_3 – soil hardness is $x_{3,0} = (2100 + 345) \cdot 2^{-1} = 1222.5 \text{ kPa}$. The variation interval is $\lambda_3 = 2100 - 1222.5 = 877.5 \text{ kPa}$, and the coded values of the third factor are $x_i = (x_3 - x_{3,0}) \cdot \lambda_3^{-1}$.

The levels of variation of the three input factors in the experimental design, in natural and coded form, are given in Table 1.

Output parameters of the study (y_i)

The output parameters derive from the purpose of the research: to establish the operational properties of the forest tillers. The output parameters in this study are operating performance, hourly and relative fuel consumption of the machines. Based on them, the optimal rotation frequency of the tiller rotor can be determined, depend-

Table 1. Levels of variation of input controllable factors – x_i in natural and coded form.

Rotor rotational frequency of the tiller – x_1		Tillage depth – x_2		Soil hardness – x_3	
Natural form	Coded form	Natural form	Coded form	Natural form	Coded form
$n = 840 \text{ min}^{-1}$	$x_1 = -1$	$a = 20 \text{ cm}$	$x_2 = -1$	$H = 345 \text{ kPa}$	$x_3 = -1$
$n = 860 \text{ min}^{-1}$	$x_1 = -0.75$	$a = 30 \text{ cm}$	$x_2 = 0$	$H = 690 \text{ kPa}$	$x_3 = -0.6$
$n = 880 \text{ min}^{-1}$	$x_1 = -0.5$	$a = 40 \text{ cm}$	$x_2 = 1$	$H = 2100 \text{ kPa}$	$x_3 = 1$
$n = 900 \text{ min}^{-1}$	$x_1 = -0.25$				
$n = 920 \text{ min}^{-1}$	$x_1 = 0$				
$n = 940 \text{ min}^{-1}$	$x_1 = 0.25$				
$n = 960 \text{ min}^{-1}$	$x_1 = 0.5$				
$n = 980 \text{ min}^{-1}$	$x_1 = 0.75$				
$n = 1000 \text{ min}^{-1}$	$x_1 = 1$				

ing on the depth of processing, the mechanical composition and the hardness of the soil. The designations of the output parameters are:

- y_1 – fuel consumption per hour; $L \cdot h^{-1}$;
- y_2 – operational performance per hour, $ha \cdot h^{-1}$;
- y_3 – relative fuel consumption per one hectare – G_o , $L \cdot ha^{-1}$.

The methods of regression analysis are used to conduct the experimental research and statistical processing of the results (Vuchkov and Stoyanov 1986). Through them, it is possible to establish the influence of various input factors on the studied parameters, to build mathematical models for predicting these parameters as response functions, and to find their optimal values. For this purpose, a three-factor experiment is planned, with three manageable and controlled input factors – rotation frequency of the tiller rotor, processing depth, and soil hardness. From the preliminary studies, it was established that the change of the output parameters in relation to the input factors has a non-linear nature. This implies that the response functions expressing operational performance, hourly and relative fuel consumption should be represented by mathematical polynomials of second or higher order. Based on these hypotheses, as well as the desire to find the optimal milling cutter rotation frequency, an optimal second-order composition plan is selected, with three levels of variation – type B_3 . From preliminary observations, it was found that the appropriate mathematical model capable of adequately describing the studied object is a second-order polynomial, as given in equation (2) (Marinov and Kostov 2022).

$$\hat{y} = b_0 + \sum_{i=1}^m b_i \cdot \dot{x}_i + \sum_{i < j} b_{ij} \cdot \dot{x}_i \cdot \dot{x}_j + \sum b_{ii} \cdot \dot{x}_i^2, \quad (2)$$

where: $-1 < \dot{x}_i < 1$ are coded values of the input factors, and $i=1, 2, \dots, m$; $m=3$ is the number of the controlled factors; b_0 is the regression coefficient of the free term; b_i are the regression coefficients of the linear terms; b_{ij} are the regression coefficients of interaction between the linear terms; b_{ii} are the regression coefficients of the squared terms.

To establish the optimal rotation frequency of the tiller rotor – n_{opt} , the technical and economic criterion for finding the minimum relative fuel consumption – G_o of the studied tilling unit is used (Marinov 2019a). In the specific case, this criterion can be expressed by the relationship given in equation (3) (Marinov 2019b).

$$n_{opt}: G_o = y_3 \rightarrow \min. \quad (3)$$

Results and Discussion

The results of the experimental study were used to develop regression mathematical models to define the studied output parameters of the forestry tiller for soil preparation, as well as to determine their optimal indicators. The experimental results obtained are subjected to statistical processing with specialised regression analysis software QstatLab-6 (1992–2025). As a result, regression equations were obtained to determine the studied output parameters, as functions of the response of the input factors – rotor speed, tillage depth and soil hardness. After excluding the insignificant coefficients from them, regression equations were obtained to determine the operational hourly performance, hourly and relative fuel consumption. The significance test of the regression coefficients b_i , b_{ij} and b_{ii} was determined at a significance level $\alpha = 0.05$.

To determine the hourly fuel consump-

tion (y_1), the operational performance (y_2), and the relative fuel consumption (y_3) of the studied forestry tiller for soil preparation in open areas, depending on the mill-

ing cutter rotation frequency (x_1), the processing depth (x_2), and the soil hardness (x_3), the regression equations (4), (5), and (6) were obtained.

$$y_1 = 43.9 + 11.36 \cdot x_1 + 10.03 \cdot x_2 + 4.63 \cdot x_3 - 0.12 \cdot x_1 \cdot x_1 + 0.32 \cdot x_2 \cdot x_2 - 1.83 \cdot x_3 \cdot x_3 + 1.93 \cdot x_1 \cdot x_2 + 1.76 \cdot x_1 \cdot x_3, \text{ L} \cdot \text{h}^{-1} \quad (4)$$

$$y_2 = 0.102 + 0.03 \cdot x_1 - 0.01 \cdot x_2 - 0.01 \cdot x_3 - 0.007 \cdot x_1 \cdot x_1 + 0.006 \cdot x_3 \cdot x_3 + 0.002 \cdot x_1 \cdot x_2 - 0.003 \cdot x_2 \cdot x_3 + 0.001 \cdot x_1 \cdot x_3, \text{ ha} \cdot \text{h}^{-1} \quad (5)$$

$$y_3 = 426 - 26 \cdot x_1 + 148 \cdot x_2 + 103 \cdot x_3 + 40 \cdot x_1 \cdot x_1 + 16 \cdot x_2 \cdot x_2 - 26.8 \cdot x_3 \cdot x_3 - 36 \cdot x_1 \cdot x_2 + 41 \cdot x_2 \cdot x_3 - 24.3 \cdot x_1 \cdot x_3, \text{ L} \cdot \text{ha}^{-1}. \quad (6)$$

The adequacy check of the obtained models and their suitability for predicting the studied output parameters was assessed using the multiple correlation coefficient R and the F-test of the Fisher distribution. The results of the ANOVA analysis of the regression models y_i are presented in Table 2.

The analysis of variance of the obtained results shows that the R multiple

correlation coefficients are significant and the obtained models are adequate. The values of the F -distribution are greater than the table values determined at the corresponding degrees of freedom, in which the condition $F > F_{\text{table}}(\alpha, v_1, v_2)$ is fulfilled. The values of the squares of the multiple correlation coefficients R^2 are close to 1, which indicates that according to the principles of variance analysis,

Table 2. ANOVA analysis of the regression models y_i from the three-factor experiment for soil preparation of open areas with a FAE SSM/HP 250 forestry tiller.

Source	Sum of squares	Degrees of freedom	Mean squared error	F	P
y_1 – Hourly fuel consumption, $\text{L} \cdot \text{h}^{-1}$					
Model	10702.97876	8	1337.87235	8922.81750	0
Residual	10.79556	72	0.14994		
Total	10713.77432	80			
$T(0.025, 72) = 1.99346$; $F_{\text{table}}(0.050, 8, 72) = 2.06983$; Residual St. Dev = 0.38722; $R^2 = 0.99899$; $R_{\text{adj}}^2 = 0.99888$; PRESS = 14.30689; $R^2(\text{pred}) = 0.99866$					
y_2 – Operational performance, $\text{ha} \cdot \text{h}^{-1}$					
Model	0.446623	8	0.055828	3094.51277	0
Residual	0.001299	72	0.000018		
Total	0.447922	80			
$T(0.025, 72) = 1.99346$; $F_{\text{table}}(0.050, 8, 72) = 2.06983$; Residual St. Dev = 0.013432; $R^2 = 0.9971$; $R_{\text{adj}}^2 = 0.99678$; PRESS = 0.01758; $R^2(\text{pred}) = 0.99607$					
y_3 – Relative fuel consumption, $\text{L} \cdot \text{ha}^{-1}$					
Model	181683.4512	9	2018.70501	531.46299	0
Residual	2696.8587	71	3.79839		
Total	184380.3098	80			
$T(0.025, 71) = 1.99394$; $F_{\text{table}}(0.050, 9, 71) = 2.01462$; Residual St. Dev = 1.9489; $R^2 = 0.98537$; $R_{\text{adj}}^2 = 0.98352$; PRESS = 425.03114; $R^2(\text{pred}) = 0.97695$					

all significant influences in the resulting models have been accounted for. The values of the squares of the coefficients of determination of the predicted values $R^2(\text{pred})$ are in the range $0.977 \div 0.999$, which means that the proposed models are suitable for predicting the studied parameters in the confidence interval up to 0.95. From the analysis conducted, it can be concluded that the regression models in equations (4), (5) and (6) are adequate and can be used with sufficiently high accuracy to determine the operational performance and fuel consumption of forest tillers depending on the rotation frequency of the tiller, the mechanical composition and hardness of the soil and tillage depth.

From the obtained models in figures 2, 3 and 4, graphical dependencies have been depicted to determine the operating performance, hourly and relative fuel

consumption of a FAE SSM/HP 250 forestry tiller, powered by a Fendt 939 Vario tractor, with rated power $N_{\text{nom}} = 265 \text{ kW}$, depending on the rotor rotation frequency of the tiller, tillage depth, mechanical composition and soil hardness.

From the graphical dependencies depicted in figures 2 and 3, it can be established that with an increase in the rotor rotation frequency, the hourly fuel consumption and the hourly operational performance of the forestry tiller increase proportionally. As the depth of tillage and the hardness of the soil increase, the hourly fuel consumption naturally increases and performance decreases. However, the relative fuel consumption has different development dynamics, which is evident from Figure 4. For example, with deeper processing up to 40 cm of heavy clay soils with a hardness of $H = 2100 \text{ kPa}$,

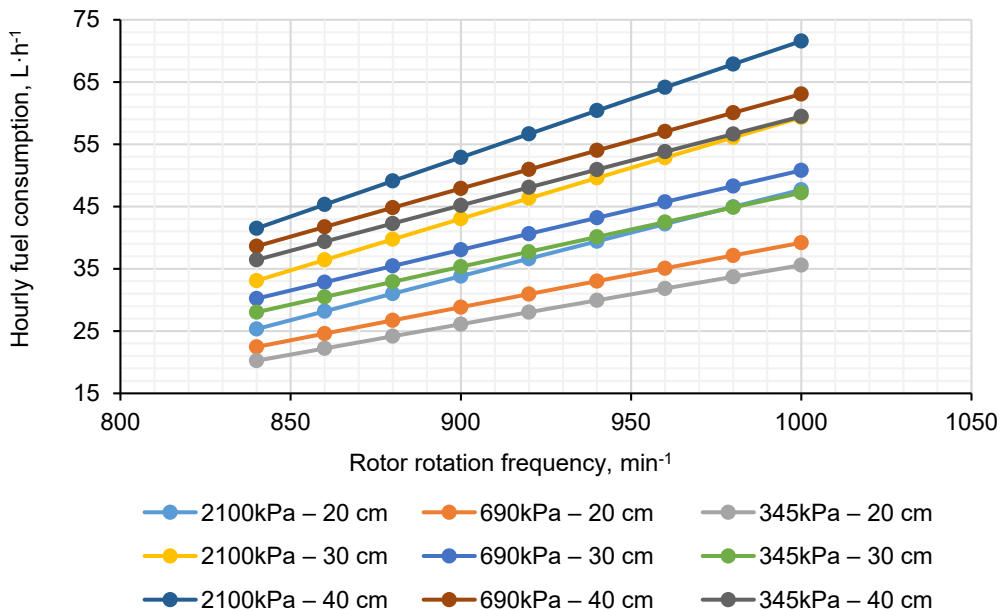


Fig. 2. Variation of the hourly fuel consumption of a forestry tilling unit for soil preparation in open areas depending on the rotor rotation frequency, tillage depth and soil hardness.

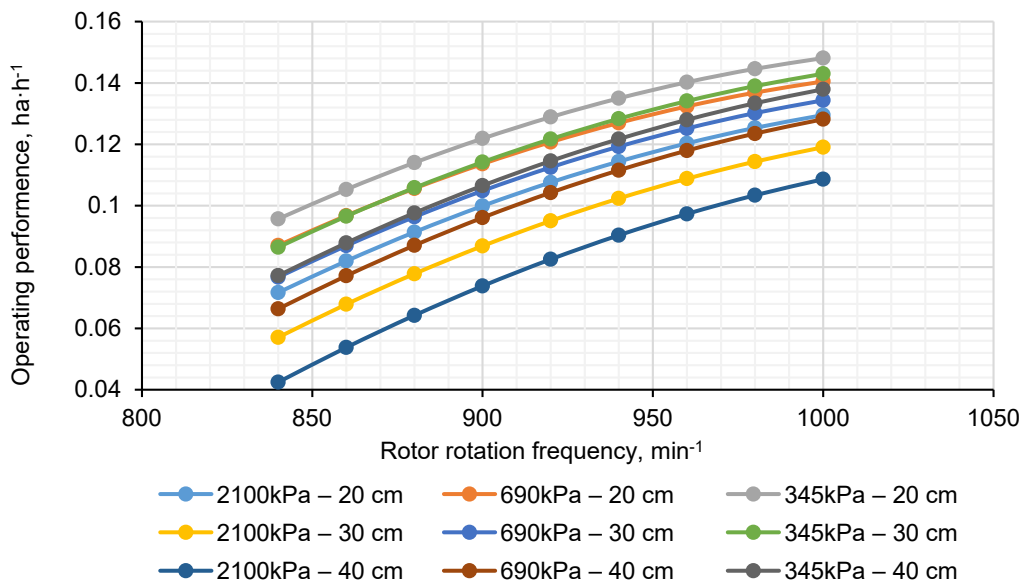


Fig. 3. Variation of the operational performance of a forestry tilling unit for soil preparation in open forest areas, depending on the rotor rotation frequency, tillage depth and soil hardness.

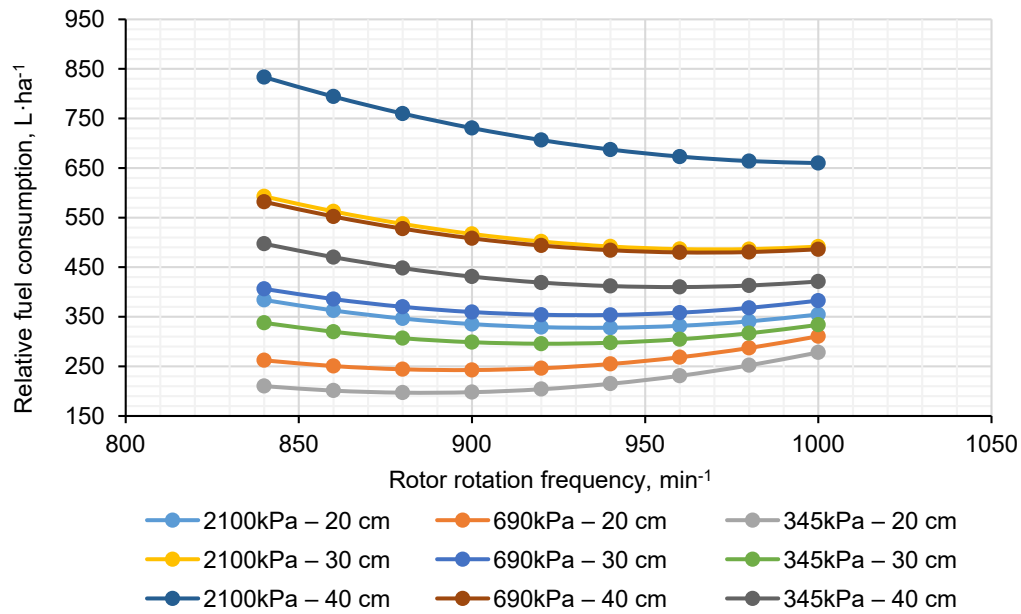


Fig. 4. Variation of the relative fuel consumption of a forestry tilling unit for soil preparation in open forest areas, depending on the rotor rotation frequency, tillage depth and soil hardness.

the relative fuel consumption decreases throughout the entire study interval of the milling cutter rotation frequency to $n = 1000 \text{ min}^{-1}$, without being able to reach its minimum. This means that when working on heavier soils and at greater processing depths, the tillers must operate at a higher rotor rotation frequency – above $n \geq 1000 \text{ min}^{-1}$ and a peripheral speed of the working knives – above $v_o \geq 35 \text{ m} \cdot \text{s}^{-1}$. By reducing the tilling depth to $a = 30 \text{ cm}$, the minimum relative fuel consumption is achieved at a rotor rotation frequency $n \approx 960 \text{ min}^{-1}$ and a knives speed $v_o \approx 33.6 \text{ m} \cdot \text{s}^{-1}$. At a tilling depth of $a = 20 \text{ cm}$, these values are respectively equal to: $n \approx 940 \text{ min}^{-1}$ и $v_o \approx 33.6 \text{ m} \cdot \text{s}^{-1}$.

During deeper processing of medium-heavy sandy-clay soils with hardness $H = 690 \text{ kPa}$ and working depth $a = 40 \text{ cm}$, the minimum fuel consumption is achieved at the rotation frequency $n = 940 \text{ min}^{-1}$ and knives speed of the tiller $v_o \approx 32.9 \text{ m} \cdot \text{s}^{-1}$. With shallower processing at a depth of $a = 20 \text{ cm}$, the minimum fuel consumption is achieved at a rotor rotation frequency and peripheral speed of the knives – $n \approx 900 \text{ min}^{-1}$ and $v_o \approx 31.5 \text{ m} \cdot \text{s}^{-1}$. At a maximum tillage depth of $a = 40 \text{ cm}$ on clay-sandy soils with a hardness $H = 345 \text{ kPa}$, the minimum relative fuel consumption of the tiller unit is obtained at a rotor rotation frequency of about $n \approx 930 \text{ min}^{-1}$ and knives speed $v_o \approx 32.5 \text{ m} \cdot \text{s}^{-1}$. With decreasing depth of tillage of such soils, the fuel cost also decreases, with the optimal speeds of the tiller at a working depth of $a = 30 \text{ cm}$ being respectively equal to: $n \approx 960\text{--}980 \text{ min}^{-1}$ and $v_o \approx 31.5 \text{ m} \cdot \text{s}^{-1}$. At a working depth of $a = 20 \text{ cm}$, these speeds are respectively: $n \approx 880\text{--}890 \text{ min}^{-1}$ and $v_o \approx 28.7 \text{ m} \cdot \text{s}^{-1}$.

From the obtained results, the following important conclusion can be drawn: when working deeper on heavy clay soils,

the rotation speed of the rotor of the tillers should be higher and vice versa, when working on lighter soils and at a smaller working depth – the working speeds of the rotor tillers should be lower.

To establish the optimal values of the rotor rotation frequency, at a certain tillage depth and soil hardness, objective optimisation was carried out to reveal the minimum of the relative fuel consumption – G_o . For this purpose, the surface of the objective function y_3 must acquire a minimum value, at which the condition $y_3 = G_o \rightarrow \min$ is satisfied. The optimisation process is performed by setting different values of the controllable input factor x_1 – rotor rotation frequency, at pre-set levels of the factors x_2 – tillage depth: $a = 20 \text{ cm}$, 30 cm and 40 cm , and of the factor x_3 – soil hardness: $H = 345 \text{ kPa}$, 690 kPa and 2100 kPa . The optimisation is performed using the gradient algorithm method, with a specialised computational programme QstatLab-6 (1992–2025). After processing the obtained results and revealing the minimum of the objective function y_3 , the optimal values of the variable x_1 were found with established initial, upper and lower levels of the other two factors – x_2 (Var2) and x_3 (Var3). The obtained optimal solutions for the rotor rotation frequency of the forestry tiller – x_1 (Var1) are given in Table 3.

As a result of the optimisation carried out from the three-factor experiment, it can be concluded that the minimum relative fuel consumption $G_{o\min} = 197 \text{ L} \cdot \text{h}^{-1}$, logically occurs at a smaller tilling depth $a = 20 \text{ cm}$ (lower level of the factor $x_2 = -1$), as well as in soils with a lighter mechanical composition and hardness $H = 345 \text{ kPa}$ (lower level of the factor $x_3 = -1$). The optimal rotor revolutions are obtained at $n_{\text{opt}} = 886 \text{ min}^{-1}$ (factor level $x_1 = -0.4222$). At the same working depth,

but when processing medium-heavy sandy-clay soils, with hardness $H = 1220$ kPa, with an established basic level of the factor $x_3 = 0$, the minimum fuel consumption

Table 3. Results of the optimisation of the FAE SSM/HP 250 forestry tiller for soil preparation of open forest areas with different soil hardness, at different tillage depths.

Tillage depth – $a = 20$ cm

Method: Gradient algorithm (GRAD); → regression model. y_3 (MIN); Iterations: 5000

Variable x_1 , min⁻¹: Range: [-1...1]; Variable x_2 , cm: $x_2 = -1 = \text{const} = (\text{lower level})$ Variable x_3 , kPa: $x_3 = -1 = \text{const} (\text{lower level})$.

***** Optimal solution:**

OBJ1 → regression model y_3 min

No	VAR1	VAR2	VAR3	OBJ1	Optimum
44	-0.4222	-1.0000	-1.0000	196.778	196.8
	886 min ⁻¹	20 cm	345 kPa		197 L·h ⁻¹

Variable x_1 , min⁻¹: Range: [-1...1]; Variable x_2 , cm: $x_2 = -1 = \text{const} = (\text{lower level})$ Variable x_3 , kPa: $x_3 = 0 = \text{const} (\text{initial basic level})$.

***** Optimal solution:**

OBJ1 → regression model y_3 min

No	VAR1	VAR2	VAR3	OBJ1	Optimum
42	-0.1197	-1.0000	0.0000	292.704	292.7
	910 min ⁻¹	20 cm	1223 kPa		293 L·h ⁻¹

Variable x_1 , min⁻¹: Range: [-1...1]; Variable x_2 , cm: $x_2 = -1 = \text{const} = (\text{lower level})$ Variable x_3 , kPa: $x_3 = 0 = \text{const} (\text{upper level})$.

***** Optimal solution:**

OBJ1 → regression model y_3 min

No	VAR1	VAR2	VAR3	OBJ1	Optimum
36	0.1823	-1.0000	1.0000	327.614	327.6
	934 min ⁻¹	20 cm	2100 kPa		328 L·h ⁻¹

Tillage depth – $a = 30$ cm

Method: Gradient algorithm (GRAD); → regression model. y_3 (MIN); Iterations: 5000

Variable x_1 , min⁻¹: Range: [-1...1]; Variable x_2 , cm: $x_2 = -1 = \text{const} = (\text{initial basic level})$

Variable x_3 , kPa: $x_3 = -1 = \text{const} (\text{lower level})$.

***** Optimal solution:**

OBJ1 → regression model y_3 min

No	VAR1	VAR2	VAR3	OBJ1	Optimum
36	0.0257	0.0000	-1.0000	295.566	295.6
	922 min ⁻¹	30 cm	345 kPa		296 L·h ⁻¹

Variable x_1 , min⁻¹: Range: [-1...1]; Variable x_2 , cm: $x_2 = -1 = \text{const} = (\text{initial basic level})$

Variable x_3 , kPa: $x_3 = 0 = \text{const} (\text{initial basic level})$.

***** Optimal solution:**

OBJ1 → regression model y_3 min

No	VAR1	VAR2	VAR3	OBJ1	Optimum
39	0.3282	0.0000	0.0000	421.206	421.2
	946 min ⁻¹	30 cm	1223 kPa		421 L·h ⁻¹

Variable x_1 , min⁻¹: Range: [-1...1]; Variable x_2 , cm: $x_2 = 0 = \text{const} = (\text{initial basic level})$

Variable x_3 , kPa: $x_3 = 1 = \text{const} (\text{upper level})$.

***** Optimal solution:**

OBJ1 → regression model y_3 min

No	VAR1	VAR2	VAR3	OBJ1	Optimum
46	0.6304	0.0000	1.0000	485.830	485.8
	970 min ⁻¹	30 cm	2100 kPa		486 L·h ⁻¹

Tillage depth – $a = 40$ cm**Method: Gradient algorithm (GRAD); → regression model. y_3 (MIN); Iterations: 5000**Variable x_1 , min^{-1} : Range: $[-1...1]$; Variable x_2 , cm: $x_2 = 1 = \text{const}$ = (upper level) Variable x_3 , kPa: $x_3 = -1 = \text{const}$ (lower level).***** Optimal solution:**OBJ1 → regression model y_3 min

No	VAR1	VAR2	VAR3	OBJ1	Optimum
46	0.4738	1.0000	-1.0000	409.904	409.9
	958 min^{-1}	40 cm	345 kPa		410 $\text{L} \cdot \text{h}^{-1}$

Variable x_1 , min^{-1} : Range: $[-1...1]$; Variable x_2 , cm: $x_2 = 1 = \text{const}$ = (upper level) Variable x_3 , kPa: $x_3 = 0 = \text{const}$ (initial basic level).***** Optimal solution:**OBJ1 → regression model y_3 min

No	VAR1	VAR2	VAR3	OBJ1	Optimum
52	0.7760	1.0000	0.0000	565.258	565.3
	982 min^{-1}	40 cm	1223 kPa		565 $\text{L} \cdot \text{h}^{-1}$

Variable x_1 , min^{-1} : Range: $[-1...1]$; Variable x_2 , cm: $x_2 = 1 = \text{const}$ = (upper level) Variable x_3 , kPa: $x_3 = 1 = \text{const}$ (upper level).***** Optimal solution:**OBJ1 → regression model y_3 min

No	VAR1	VAR2	VAR3	OBJ1	Optimum
36	1.0000	1.0000	1.0000	659.843	659.8
	1000 min^{-1}	40 cm	2100 kPa		660 $\text{L} \cdot \text{h}^{-1}$

$G_{o \min} = 296 \text{ L} \cdot \text{h}^{-1}$ is obtained at optimal rotor frequency $n_{\text{opt}} = 886 \text{ min}^{-1}$ (level of $x_1 = -0.4222$). When tilling heavier clay soils with hardness $H = 2100 \text{ kPa}$ (upper level of $x_3 = 1$), at the same depth $a = 20 \text{ cm}$ (lower level of $x_2 = -1$), the optimal frequency is $n_{\text{opt}} = 934 \text{ min}^{-1}$ (level of $x_1 = 0.1823$), where the minimum fuel consumption is $G_{o \min} = 328 \text{ L} \cdot \text{h}^{-1}$. As the tilling depth increases, the optimal rotor speed also increases. For example, in soil preparation of medium-heavy sandy-clay soils, with a hardness of $H = 1220 \text{ kPa}$ (basic level of $x_3 = 0$), the optimal frequency at a working depth of $a = 30 \text{ cm}$ (basic level of $x_2 = 0$) is $n_{\text{opt}} = 946 \text{ min}^{-1}$ (level of $x_1 = 0.3282$), and at a depth of $a = 40 \text{ cm}$ (upper level of $x_2 = 1$) it is $n_{\text{opt}} = 970 \text{ min}^{-1}$ (level of $x_1 = 0.6304$). The minimum relative fuel consumption at these working depths and rotor rotation speed is respectively equal to $G_{o \min} = 421 \text{ L} \cdot \text{h}^{-1}$ and $G_{o \min} = 486 \text{ L} \cdot \text{h}^{-1}$. In soil preparation of heavier clay soils with

hardness $H = 2100 \text{ kPa}$ (upper level of factor $x_3 = 1$), at working depth $a = 30 \text{ cm}$ (basic level of $x_2 = 0$) the optimal frequency of the rotor rotation is $n_{\text{opt}} = 982 \text{ min}^{-1}$ (level of $x_1 = 0.776$), and at working depth 40 cm (upper level of $x_2 = 1$), it is $n_{\text{opt}} = 1000 \text{ min}^{-1}$ (level of $x_1 = 1$). The minimum relative fuel consumption at those tilling depths and rotor rotation frequencies is respectively equal to $G_{o \min} = 565 \text{ L} \cdot \text{h}^{-1}$ and $G_{o \min} = 660 \text{ L} \cdot \text{h}^{-1}$.

The proposed regression models in equations (4), (5) and (6) can be used with sufficiently high accuracy to determine the optimal rotor speed of forestry tillers powered by tractors with a rated power in order of 265 kW , as well as to predict the operating productivity, hourly and relative fuel consumption during soil preparation of open forest areas with different mechanical composition and soil hardness from 345 kPa to 2100 kPa , at a tillage depth of 20 cm to 40 cm . The surfaces of the function of response y_3 and

the diagram of the isolines with constant values of the relative fuel consumption of a FAE forestry tilling unit with rated power $N_{\text{nom}} = 265 \text{ kW}$, during soil preparation of open forest areas with different soil hard-

ness and different tilling depths, are depicted in figures 5, 6 and 7. In these diagrams, red lines mark the lines connecting the optimum values of the rotor rotation frequency, where the minimum values of

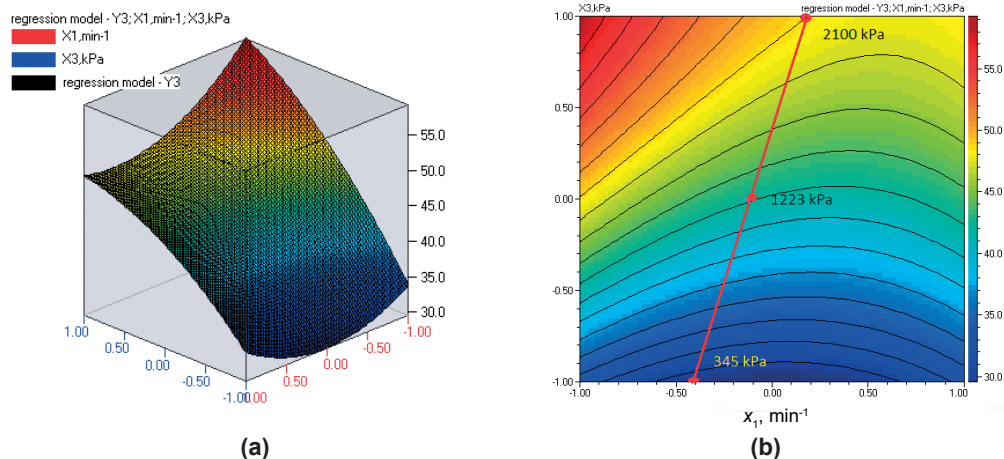


Fig. 5. Amendment of objective function y_3 – relative fuel consumption of a FAE SSM/HP 250 tiller for processing soil of different hardness, at a tillage depth of 20 cm.

Note: (a) – surface of the objective function y_3 ; (b) – diagram of isolines with constant values.

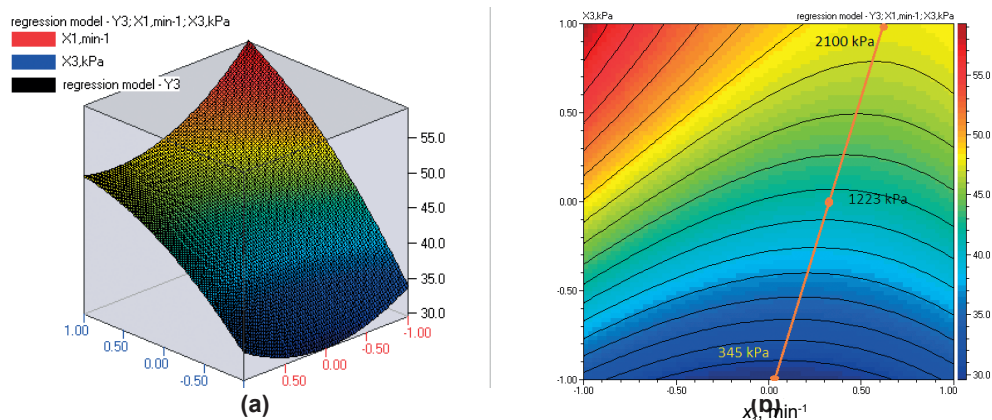


Fig. 6. Amendment of objective function y_3 – relative fuel consumption of a FAE SSM/HP 250 tiller for processing soil of different hardness, at a tillage depth of 30 cm.

Note: (a) – surface of the objective function y_3 ; (b) – diagram of isolines with constant values.

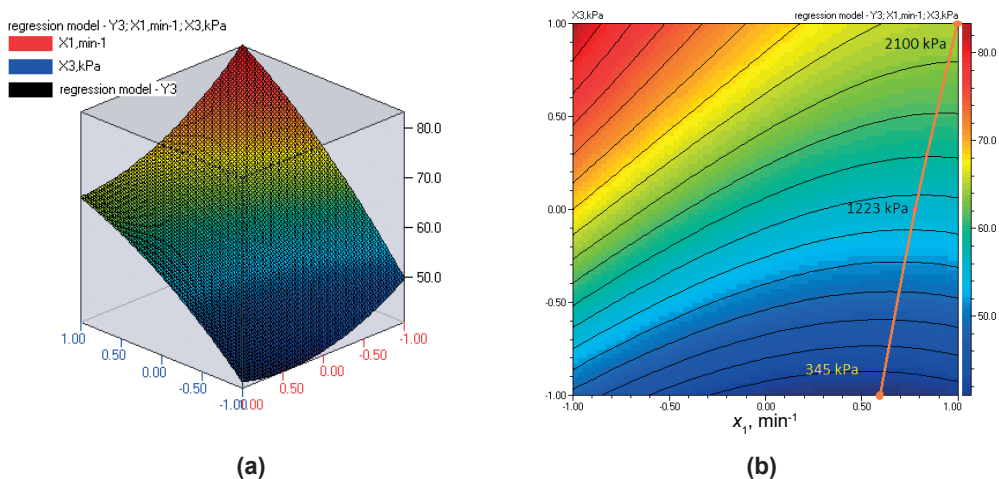


Fig. 7. Amendment of objective function y_3 – relative fuel consumption of a FAE SSM/HP 250 tiller for processing soil of different hardness, at a tillage depth of 40 cm.
 Note: (a) – surface of the objective function y_3 ; (b) – diagram of isolines with constant values.

the relative fuel consumption were found, in the main treatment of different soils, with hardness from 345 kPa to 2100 kPa, at a depth of 20 cm, 30 cm and 40 cm.

Conclusions

As a result of the experimental research conducted with the FAE SSM/HP 250 forestry tiller, driven by a Fendt 939 Vario tractor with a rated power of 265 kW, it was found that during soil preparation for afforestation of open forest areas with lighter clay-sandy soils with a hardness of $H = 345$ kPa, at a shallow processing depth of $a = 20$ cm, the minimum relative fuel consumption is $G_{o \min} = 197 \text{ L} \cdot \text{h}^{-1}$. The optimal forestry tiller rotation speed under these operating conditions is $n_{\text{opt}} = 886 \text{ min}^{-1}$. At the same tillage depth of medium-heavy sandy-clay soils with a hardness of $H = 1220$ kPa, the minimum fuel consumption at the same tiller rotation frequency $n_{\text{opt}} = 886 \text{ min}^{-1}$ is equal

to $G_{o \min} = 296 \text{ L} \cdot \text{h}^{-1}$. When preparing heavier clay soils with greater hardness $H = 2100$ kPa, at the same tillage depth $a = 20$ cm, the optimal rotation frequency of the tiller is higher – $n_{\text{opt}} = 934 \text{ min}^{-1}$, with the minimum relative fuel consumption here being equal to $G_{o \min} = 328 \text{ L} \cdot \text{h}^{-1}$.

As the depth of soil preparation increases, the optimal rotation frequency of the tiller increases. When processing medium-heavy sandy clay soils with a hardness of $H = 1220$ kPa, at a working depth of $a = 30$ cm, the optimal rotation speed of the tiller is equal to $n_{\text{opt}} = 946 \text{ min}^{-1}$, and at a working depth of $a = 40$ cm, it is already $n_{\text{opt}} = 970 \text{ min}^{-1}$. The minimum relative fuel consumption under these operating conditions is respectively equal to $G_{o \min} = 421 \text{ L} \cdot \text{h}^{-1}$ and $G_{o \min} = 486 \text{ L} \cdot \text{h}^{-1}$. When tilling the soil for afforestation of field protection forest belts on heavier clay soils with a hardness of $H = 2100$ kPa, at a tillage depth of $a = 30$ cm, the optimal rotor rotation frequency of the tiller is equal to $n_{\text{opt}} = 982 \text{ min}^{-1}$,

and at a working depth of $a = 40$ cm, it already reaches the maximum rotor rotation frequency – $n_{\text{opt}} = n_{\text{max}} = 1000 \text{ min}^{-1}$. The minimum relative fuel consumption under these production conditions is respectively equal to $G_{\text{o min}} = 565 \text{ L} \cdot \text{h}^{-1}$ and $G_{\text{o min}} = 660 \text{ L} \cdot \text{h}^{-1}$.

Functional mathematical models have been developed to determine the hourly operational performance, hourly and relative fuel consumption of a forestry milling unit for soil preparation in open forest areas, depending on the rotor revolutions frequency of the tiller, the mechanical composition and the hardness of the soil, and the tillage depth. They can be used to determine the fuel and time costs of a FAE SSM/HP 250 forestry tiller for soil preparation for forest protection belts establishment in open forest areas.

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Comparative evaluation of fruit biometric parameters of local cherry cultivars and forms

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Thematic field: Agriculture in mountainous and semi-mountainous regions

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Abstract

In 2023, during scientific expeditions in the regions of Shumen, Ruen, Kuklen, Osmar, Varna and Aytos, part of a project for preservation of local genetic resources, nine local cherry cultivars and forms were discovered. The aim afterwards was to make a pomological description and identify the cultivars, as well as determining biometric parameters and ripening dates. Specimens were mapped with GPS coordinates in the project's database, the ripening period of the fruits was determined, as well as their pomological characteristics. Biometric analyses of fruit and stones were performed and compared to the standard cultivars 'Van' and 'Bigarreau Burlat'. The earliest ripening fruits are of specimen SS-08 on 8 June, and the latest ripening are of SM-29 is on 26 June. Specimen SS-06 had the highest fruit weight 6.09 g from the discovered cherry forms, and SM-29 had the lowest fruit weight 1.31 g. The stone weight ranged from 0.15 g in SM-29 to 0.36 g in SS-06. The shortest stalk was measured for SS-09 at 22 mm and the longest for SS-08 at 62 mm.

Key words: biometric analyses, fruit, genetic resources, local forms, old cherry cultivars, stone.

Introduction

Genetic resources are valuable heritable material possessing the genetic information of given cultivars and their parent.

Their conservation and preservation are important as climate change and genetic erosion lead to their extinction (Thor-

mann and Engels 2015, Salgotra and Chauhan 2023). In different countries, local cultivars possess a variety of valuable agronomic and biological characteristics that represent a valuable genetic resource for breeding activities in the creation of new cultivars (Kirca and Ayygün 2024, Perju et al. 2025).

The creation of new cultivars in the world is increasing annually and their introduction into our country is inevitable. However, their adaptability poses risks to the growing conditions in our country. In Bulgaria, there is a wide variety of native species and wild forms, which are a valuable source of genetic material possessing resistance to abiotic and biotic stress factors (Vasilev et al. 2019). For the effective use of local cultivars in the breeding programs, it is necessary to conduct complex studies aimed at identifying their positive biological and agronomic characteristics. This will support the precise selection and formation of appropriate parental combinations leading to the creation of new varieties with optimal economic qualities (Reim et al. 2023). By using them in breeding, as donors of genetic material, resistant cultivars are created (Zhivondov 2012). For this, a team of scientists from the Fruit Growing Institute – Plovdiv (FGI) focused on searching for old cultivars in different regions of the country, completed series of expeditions across the country though out extended period of time. In this way, representatives of many local cultivars and forms with valuable characteristics were found and mapped with GPS coordinates.

The aim of the present study was pomological description of the discovered cherry forms and identified cultivars, linking the biometric parameters and ripening dates to exact GPS coordinates of the discovered specimens.

Materials and Methods

During a survey in the area around Shumen city (43°15'58.7" N, 26°56'19.2" E) and city of Varna (43°13'09.7" N, 27°45'25.5" E), one cultivar and several

cherry forms were found (Table 1).

Also, in the area around town of Kuklen (42°01'33.5" N, 24°44'40.7" E) and village of Ruen (42°00'49.3" N, 24°48'22.2" E) the cultivars 'Ruenska belitsa' and one local cherry form were found. A GPS coordinate map has been compiled, and the fruit ripening dates were visually determined. Pomological descriptions were made. The biometric parameters of fruit height, thickness and width were measured using a digital calliper. Fruit and stone weight were determined by measuring 25 fruits using an electronic balance. The results were statistically processed using the method developed by David B. Duncan (Duncan 1955, Harter 1960). The software used was R version 3.1.3, in combination with RStudio version 0.98 and agricolae package version 1.2-2 (Mendiburu 2015).

Results and Discussion

Through the compilation of pomological descriptions of the cultivars and forms discovered, the research team aim at specimen identification, conservation and selection of valuable traits usable in future breeding work.

The located cherry cultivars and forms ripen in June, in the first ten days ripen the fruits of SS-08 locally referred to as 'Byala cheresha', SS-06 identified as 'Cherna osmarka' and SS-05 not yet identified. Maturing in the second ten days are SM-04 identified as 'Ruenska belitsa' and SS-09, SS-10 and SS-11 that not identified.

Last to ripen at the end of the month were the fruits of SM-29 grafted local form of bird cherry and SS-07 referred to as 'Starata cheresha' (Table 1). The fruit shape is oblate in SM-04, SM-29, SS-08 and SS-11. Reniform shaped were SS-06, SS-07, SS-09 and SS-10. Cordate shape

Table 1. GPS coordinates and ripening date of cherry forms and cultivars.

Sample No	Origin of the sample		Date of sampling	Ripening time at the originating region	Notes
	Coordinates	Locality			
SM-04	42°00'49.3" N 24°48'22.2" E	Ruen (Bogorod-ichno) village	17.06.2021	14 Jun	Identified as 'Ru-enska belitsa'
SM-29	42°01'33.5" N 24°44'40.7" E	Kuklen Monastery 'St. St. Kozma and Damyan'	26.06.2021	26 Jun	Local form bird cherry grafted on <i>P. mahaleb</i>
SS-05	43°12'38.8" N 26°50'43.6" E	Han Krum vil-lage (road to Osmar)	09.06.2023	10 Jun	N/a
SS-06	43°13'01.7" N 26°50'22.5" E	Osmar village	09.06.2023	9 Jun	Identified as 'Cherna osmar-ka'
SS-07	43°12'07.0" N 26°57'14.1" E	Marash village	10.06.2023	24 Jun	Referred to as 'Starata chere-sha'
SS-08	43°15'58.7" N 26°56'19.2" E	Shumen city	10.06.2023	8 Jun	Referred to as 'Byala cheresha'
SS-09	43°13'09.7" N 27°45'25.5" E	Varna city (sub-urbs)	11.06.2023	17 Jun	N/a
SS-10	42°41'14.9" N 27°08'46.4" E	Aytos city (high-way to Karnobat)	11.06.2023	15 Jun	N/a
SS-11	42°41'28.1" N 27°09'47.1" E	Aytos city (high-way to Karnobat)	11.06.2023	12 Jun	N/a

had only specimen SS-05. The different degrees of basic colouration of the fruit skin in the cultivars and forms observed varied from dark red and light red to yellow. SS-06, SS-10 and SS-11 have dark red colouring. Red colour was identified only in SS-07. Light red skin colouration was present in SM-29 and SS-09. Fruit ground (basic) colour for SM-04, SS-05 and SS-08 is yellow. Bicoloured cherries are SM-04 and SS-05 with light red as cover fruit colour (Table 2).

Fruit weight is a heritable trait determining fruit size and the first indicator attracting the consumer (Nesheva et. al. 2020, Sejfić et. al. 2023). The weight of the cultivars studied ranged from 1.31 g for SM-29 to 9.59 g for the standard 'Van', and the difference between the different

cultivars was statistically proven (Table 3). With similar values to 'Bigarreau Burlat' for the fruit weight was found for cherry SS-06 with an average mass of 6.09 g. Medium sized fruits with mass between 4–5 g were reported, such specimens are SS-11 and SS-10, SM-04, SS-07, SS-08 and SS-09. A smaller value was reported for SS-05 – 3.83 g. The greater the mass of the fruits the greater their dimensions for fruit height, width and thickness (Table 4).

The smallest average stone value was observed in the same forms having the smallest fruit mass SM-29 – 0.15 g and SS-05 – 0.19 g. An average mass between 0.25 g and 0.27 g have SM-04, SS-08, SS-09 and SS-11. The fruits of the

Table 2. Pomological characteristics of cherry fruits.

Sample No	Fruit shape	Fruit colour – basic/ground	Fruit colour – cover
SM-04	Oblate	Yellow	Light red
SM-29	Oblate	Light red	N/a
SS-05	Cordate	Yellow	Light red
SS-06	Reniform	Dark red	N/a
SS-07	Reniform	Red	N/a
SS-08	Oblate	Yellow	N/a
SS-09	Reniform	Light red	N/a
SS-10	Reniform	Dark red	N/a
SS-11	Oblate	Dark red	N/a

Table 3. Weight of fruit and stone length of stalk length.

Sample No	Fruit weight, g	Stone weight, g	F/S ratio, %	Stalk length, mm
SM-04	4.64 ef	0.27 c	5.82	40.21 c
SM-29	1.31 i	0.15 e	11.24	23.36 e
SS-05	3.83 h	0.19 d	4.90	38.70 cd
SS-06	6.09 c	0.36 a	5.98	38.42 cd
SS-07	4.92 e	0.33 b	6.73	37.47 cd
SS-08	4.31 fg	0.27 c	6.31	62.61 a
SS-09	4.08 gh	0.25 c	6.24	22.88 e
SS-10	5.85 cd	0.34 ab	5.82	47.09 b
SS-11	5.62 d	0.27 c	4.76	35.67 d
'Bigarreau Burlat'	6.98 b	0.33 b	4.67	24.63 e
'Van'	9.59 a	0.34 ab	3.56	22.23 e

Note: Different letters in the same column indicate a significant difference at $p = 0.05$.

two reference cultivars 'Bigarreau Burlat' and 'Van', SS-07 and SS-06 had an average weight between 0.33 g to 0.36 g. The obtained biometric dimensions of height, width, and stone thickness were within normal limits corresponding to the obtained mass (Table 5). One of the important indicators is the ratio of fruit flesh to stone. From the cultivars and shapes examined, we find that these are within the acceptable range. In a study Blazkova (1987) found that the ratio of stone to fruit fresh weight ranged from 3.7 to 8.4 %. The percentages obtained in our study fall within these limits. The

exception is SM-29 where the percentage obtained exceeds the upper limit of 11.24 %.

Comparing the stalk length of all nine cherry forms and cultivars found, we find that only SS-09 and SM-29 are close in size to those of the two standards. On average, the stem values are longer in the range of 35 mm to 40 mm. The longest stalk was measured in SS-08 'Byala cheresha' (White cherry) at 62.61 mm.

Figure 1 shows photographs of cherry cultivars and forms found. Variation in fruit colour, fruit size, and stalk length can be observed.

Table 4. Biometric characteristics of cherry fruits.

Sample No	Fruit height, mm	Fruit width, mm	Fruit thickness, mm
'Bigarreau Burlat'	22.52 b	23.27 b	19.61 b
SM-04	20.83 c	19.79 ef	17.19 f
SM-29	12.29 h	13.11 i	11.85 i
SS-05	16.77 g	18.16 h	15.56 h
SS-06	20.96 c	21.78 c	18.17 de
SS-07	19.41 d	20.08 e	17.85 e
SS-08	18.53 e	18.89 g	15.53 h
SS-09	17.55 f	19.29 fg	16.61 g
SS-10	19.00 de	21.80 c	19.02 c
SS-11	18.91 de	20.80 d	18.45 d
'Van'	24.24 a	26.92 a	22.04 a

Note: Different letters in the same column indicate a significant difference at $p = 0.05$.

Table 5. Biometric characteristics of cherry stone.

Sample No	Stone height, mm	Stone width, mm	Stone thickness, mm
'Bigarreau Burlat'	11.88 a	6.79 d	8.65 c
SM-04	10.90 c	5.88 g	7.92 d
SM-29	7.30 g	5.18 h	6.65 f
SS-05	8.66 f	6.34 e	7.61 e
SS-06	11.43 b	7.54 a	9.35 a
SS-07	11.07 bc	7.02 bc	8.99 b
SS-08	11.07 bc	6.09 f	8.41 c
SS-09	9.85 de	6.85 cd	7.93 d
SS-10	10.22 d	7.43 a	9.09 b
SS-11	10.24 d	6.92 bcd	8.64 c
'Van'	9.70 e	7.07 b	9.03 b

Note: Different letters in the same column indicate a significant difference at $p = 0.05$.

Conclusions

All cherry specimens from local forms and old cultivars discovered during this research have been mapped with exact GPS coordinates linked with pomological description for future exploitation and preservation. The search, study and preservation of such specimens is continuous

and a priority for the FGI. This will contribute to maintaining the Bulgarian heritage and culture.

Despite of their small fruit size, the old local cherry cultivars have diverse valuable qualities linked both to local traditions and biodiversity, vital to adaptation in ever changing climate and consumer preferences.



SS-05



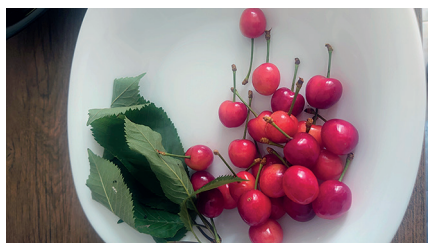
SS-06



SS-07



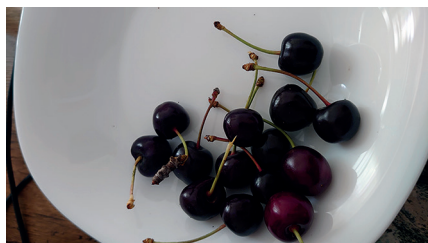
SS-08



SS-09



SS-10



SS-11



'Ruenska belitsa'

Local form bird cherry grafted on *P. mahaleb*

Fig. 1. Fruits of cherry forms and cultivars found.

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Accumulation of copper, mercury and arsenic in skeletal muscles, liver and kidneys of representatives of Cervidae family – a review

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Thematic field: Wildlife conservation and management

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Abstract

Representatives of Cervidae family are important bioindicators of the environmental pollution caused by various heavy metals. Significant amounts of toxic elements can accumulate in tissues and organs, particularly in the liver and kidneys. The study of accumulation levels can provide valuable information about the state of ecosystems and potential risks to human health, especially for areas with developed intensive industrial activity or proximity to pollution sources. The aim of this review is to summarise and compare reported data (2000–2024) on copper (Cu), mercury (Hg), and arsenic (As) accumulation in three edible organs (muscle, liver, and kidney) of the following members of Cervidae family: Roe deer (*Capreolus capreolus* L., 1758), Red deer (*Cervus elaphus* L., 1758), and Fallow deer (*Dama dama* L., 1758), and to discuss bioaccumulation trends and the potential health risks posed by the consumption of these products. The literature findings were compared with the established permissible limits according to European and international regulations. The possible health hazards associated with the consumption of meat and by-products, accumulation pathways, and organs affected by toxic damage were also examined. This information is essential for assessing the safety of wild game meat and by-products for human consumption, as well as for developing effective strategies for environmental protection and management of wild populations. In most studies, the reported levels of Cu, Hg, and As in

deer meat, liver, and kidneys did not exceed the established safety thresholds. However, isolated cases of elevated concentrations, especially in the liver and kidney tissues from polluted areas, underscore the necessity of regular monitoring and cautious consumption.

Key words: game meat, game organs, health hazard, heavy metals accumulation.

Introduction

Importance of Cervidae representatives as game hunting targets in Europe

Cervidae family represents a world-wide distributed diverse group of herbivorous ungulates that plays an important role in the management and preservation of wild ecosystems and farm breeding practices (Tanentzap et al. 2012, Zorkóczy et al. 2025). In Europe, deer hunting has a rich history and ancient cultural traditions (Albarella and Aniceti 2024). It has advanced under the influence of biological, ecological, and cultural-ethical factors (Milner et al. 2006, Fischer et al. 2013) and is no longer performed mainly for self-preservation and sustenance. Deer hunting plays a significant role in wildlife management, contributing to rural economies, recreational activities, educational practices, and cultural heritage (Burbaité and Csányi 2010, Fischer et al. 2013, Bicho et al. 2024).

Amongst the most significant Cervidae representatives in northern Europe are Roe deer (*Capreolus capreolus* L., 1758), Red deer (*Cervus elaphus* L., 1758), and Fallow deer (*Dama dama* L., 1758) (Ramirez 2021), all of which are considered primary targets for game hunting (Mattioli 2019, Jarnemo 2023). Therefore, it is essential to gain a thorough understanding of the ecological and economic relevance of these keystone species as well as the impact of both human activ-

ities and environmental factors on their ecosystems.

Roe, Red, and Fallow deer are widely distributed in various landscapes across the continent and possess considerable ecological, economic, and cultural significance (Burbaité and Csányi 2009, Apollonio et al. 2010, Gryz et al. 2024). They function as prey and food source for predators and scavengers. They also affect plant species and soil conditions through their grazing and browsing habits (Hagge et al. 2019, Schwegmann et al. 2023, Ruco and Marucco 2025). Human activities, such as hunting, species reintroduction, and various land-use practices (agricultural fields, residential areas, roads, industrial sites, etc.) can influence animal density and distribution, often exceeding the impact of natural predators (Lone et al. 2015, van Beeck Calkoen et al. 2023, Takada and Nakamura 2024).

While Fallow deer are not indigenous to all parts of Europe and were originally introduced from the Mediterranean region (Masseti 1996), nowadays, this species is well established as one of the most common cervid species in Europe and the most widespread in the one world (Chapman and Chapman 1980).

The economic importance of Cervidae species as game hunting objects is considerable (Bicho et al. 2024). They contribute significantly to rural economies and the hunting sector through venison production (Viganò et al. 2019) and sport hunting (McGuigan 2017). Diverse hunt-

ing activities produce direct economic benefits through hunting fees, equipment sales, hunting tourism, and other services (Kupren and Hakuć-Błażowska 2021).

Meat composition and quality

The quality and composition of meat from various deer species have gained considerable attention in recent years. Human consumption of deer meat depends primarily on its quality, taste, and health-promoting properties (Dransfield 2003; Wiklund et al. 2014). Venison is valued for its specific flavour and nutritional benefits, such as its distinct chemical composition, high protein levels, low fat content, and favourable fatty acid profile (Daszkiewicz et al. 2009, Bureš et al. 2015, Maggiolino et al. 2019). This makes it an increasingly popular choice for culinary connoisseurs and health-conscious consumers, especially when sourced from free-range or sustainably managed populations (Wiklund et al. 2014).

1. Protein content and amino acid composition

Roe deer meat is known for its high protein content, with an average of 23 %, compared to 22 % in Fallow deer and 21.7 % in Red deer (Blaška et al. 2016). It is influenced by factors such as age, sex, and the specific muscle analysed (Strazdiņa et al. 2013, Milczarek et al. 2021). The approximate protein content is also higher than that of domestic livestock species: 20.83 % in lean beef, 20.43 % in mutton, and 18 % in goat meat (Blaška et al. 2016). Additionally, the amino acid profile of Roe deer meat demonstrates a high level of essential amino acids, particularly tryptophan and sulphur-containing amino acids, which can be higher than those

found in meat from other comparable livestock such as goats (Cygan-Szczegielniak and Janicki 2012).

Red deer meat is characterised by a high total protein content and relatively low total collagen content (Daszkiewicz et al. 2009). Its quality characteristics depend on the analysed muscle, age, and sex of the animal (Daszkiewicz et al. 2009, Lorenzo et al. 2019, Maggiolino et al. 2019). The amino acid profile of Red deer meat is well balanced, with glutamic acid (approximately 16 % of total amino acids), followed by lysine and aspartic acid (approximately 10 % TAA) being the major components. The proportion of essential amino acids matches human dietary requirements; lysine is reported to be the most abundant, followed by leucine and arginine (together covering approximately 52 % of the total essential amino acids) (Lorenzo et al. 2019).

Fallow deer meat typically has a high protein content (20.04–23.1 %) (Cawthorn et al. 2020), comparable to other game meats, and higher than certain conventional lean meats, such as rabbit (21.4 %) and chicken (20.1 %) (Nistor et al. 2013). Studies have demonstrated that intramuscular fat content of Fallow deer meat can be very low, ranging from 1.28 % to 2.66 % (Švrčula et al. 2019) and even lower than 1 % (Volpelli et al. 2002), which is consistent with contemporary dietary recommendations.

2. Fatty acids profile

Regarding fatty acid composition, Roe deer meat is characterised by a higher proportion of unsaturated fatty acids, notably monounsaturated (MUFA) and polyunsaturated (PUFA) (Cygan-Szczegielniak and Janicki 2011, Daszkiewicz and Mesinger 2018). Research indicates

that the ratio of unsaturated to saturated fatty acids is beneficial for cardiovascular health, with Roe deer meat having a more favourable fatty acid composition than livestock meat (Cygan-Szczegieliński and Janicki 2011). This includes marked levels and favourable ratios of essential ω -6 and ω -3 (ω -6: ω -3 = 2.07) (Strazdiņa et al. 2013), which positively influence human health (Milczarek et al. 2021).

In terms of lipid composition, Red deer meat is relatively low in fat, with a favourable fatty acid profile, characterised by a high proportion of unsaturated fatty acids, particularly ω -3 and ω -6 (Daszkiewicz and Mesinger 2018, Kasprzyk et al. 2022). The content of alpha-tocopherol in the meat of Red deer is also significant, around $5.9 \mu\text{g}\cdot\text{g}^{-1}$ of meat, and the sum of all vitamin E homologues ($9.9 \mu\text{g}\cdot\text{g}^{-1}$ of meat) is within the value necessary for adequate antioxidant protection to venison ($7\text{--}9 \mu\text{g}\cdot\text{g}^{-1}$ of meat) (Quaresma et al. 2012).

The fatty acid composition of Fallow deer meat is notable for its balance of PUFA and MUFA, including both ω -3 and ω -6. Approximately 97 % of ω -3 long-chain PUFA are derived from eicosapentaenoic (EPA), docosapentaenoic (DPA), and docosahexaenoic acid (DHA) (Mexia et al. 2020), providing essential fatty acids that are increasingly recognised for their beneficial effects on cardiovascular health (Kilar and Kasprzyk 2021).

3. Mineral profile

The mineral composition of deer meat contributes to its nutritional value. It is rich in essential minerals, such as iron, zinc, calcium, and magnesium, which are crucial for numerous body functions, including the immune response, metabolic processes, and structural integrity of

bones and teeth. Iron in deer meat is predominantly in the haem form, which has better absorption and bioavailability than the non-haem iron found in plant sources (Kasprzyk and Kilar 2023).

Beneficial and harmful effects of deer organ meat consumption

In addition to deer meat, certain visceral organs, such as liver, kidneys, heart, and lungs, are also consumed and valued for their rich nutritional content and culinary versatility. However, edible offal from Roe, Red, and Fallow deer has both beneficial and negative health effects when used for human consumption. Data on deer offal consumption are scarce. For cervids, a Finnish survey of moose hunters reported that 69 % consumed liver and 23 % kidneys (Vahteristo et al. 2003). An Italian survey likewise found that meat, liver and heart were preferred items, with typical consumption of around 100–200 g portions several times per month (Ferri et al. 2017). These findings suggest that, while offal consumption in the general population could be considered uncommon, hunters and their families have substantially higher intakes, which is relevant for exposure assessments.

The beneficial effects of deer liver and kidney consumption include nutritional value and health-promoting properties. The liver is recognised for its high concentrations of vitamins, particularly A and E, and essential minerals such as iron, copper, and zinc, which play vital roles in haematopoiesis, various metabolic processes, immune function, and stress resistance (Tremain-Boon et al. 2002; Rodríguez-Estival et al. 2011a, 2011b; Jarzyńska and Falandysz 2011; Cygan-Szczegieliński 2021). Furthermore, deer liver and kidneys contain antioxidant components that may con-

tribute to combating oxidative stress (Pilarczyk et al. 2009, 2011; Jarzyńska and Falandysz 2011). Deer meat and edible offal have a favourable fatty acid composition, often high in beneficial ω -3 fatty acids, with positive effects on the cardiovascular system and cognitive health (Zomborszky and Husvéth 2000, Razmaité et al. 2020).

Although the consumption of deer liver and kidneys can bring significant nutritional benefits, it also poses potential health risks. Understanding the most common potentially harmful effects, such as heavy metal accumulation and zoonotic diseases, is essential for ensuring human health through safe consumption.

Wild deer may act as reservoirs and vectors of zoonotic pathogens and transmit pathogens to humans through various pathways. Consumption of contaminated food, especially meat, plays an important role in the spread of certain bacterial diseases, including *Yersinia pseudotuberculosis* (Pfeiffer, 1889), *Salmonella* spp., *Escherichia coli* O157 (Migula, 1895), *Brucella abortus* (Buchner, 1897), *Brucella ovis* (Buddle, 1956), *Brucella suis* (Huddleson, 1929), *Erysipelothrix rhusiopathiae* (Migula, 1900)), helminth parasites such as *Echinococcus granulosus* (Batsch, 1786), and protozoan infections, notably *Toxoplasma gondii* (Nicolle & Manceaux, 1908) and *Sarcocystis* spp. (Böhm et al. 2007). Viral infections, such as those caused by Hepatitis E virus, have been detected in deer livers, posing an additional risk to consumers (Serracca et al. 2015, Thiry et al. 2017, Fonti et al. 2022). Instances of human illness associated with the consumption of undercooked or raw deer meat and offal underscore the importance of the proper sourcing, handling, and thermal processing of game products.

A major issue associated with consuming deer organ meat is the possible accumulation of heavy metals and metalloids, particularly in the liver and kidneys, which play vital roles in detoxification (Jota Baptista et al. 2024). Cervid species are susceptible to accumulating various environmental pollutants in their organisms, particularly in habitats near industrial sites (Bąkowska et al. 2024).

Wild deer meat consumption

In contemporary times, the consumption of venison demonstrates a notable increase, not only among hunters and their families, but also among health-conscious consumers and culinary enthusiasts, due to its health benefits and distinctive flavour (Niewiadomska et al. 2020). This trend is especially evident in regions with well-established deer hunting and/or farming practices, as well as in those with long cultural traditions and hunting heritage.

As an emerging segment of the meat industry, deer meat has demonstrated a consistent rise in global trade to approximately two million tonnes each year (Serrano et al. 2019). Nevertheless, the availability of deer meat on the market varies widely across European countries, depending on national hunting regulations, processing infrastructure, and supply chains (Needham et al. 2023). Consequently, overall consumption remains modest – generally between 0.2 and 1.1 kg per capita per year – with only a small share of consumers (2–4 %) eating it regularly (Tomić Maksan et al. 2025).

Findings from a probabilistic approach, estimating the consumption of meat and products derived from hunting, especially by hunters and their relatives in Spain, indicate an average of 6.87 kg of game meat per capita per year, which increas-

es to 8.57 kg per person per year when including also the processed meat products. Within those numbers, individually the Red deer meat consumed accounts for 2.28 kg per person per year, which is reported to be in line with the mean consumption of deer meat in Europe (2–3 kg per person per year) (Sevillano-Morales et al. 2018). Wild ungulate meat consumption in some Italian regions has significantly increased, especially in hunter families (1.0–4.0 kg per capita per year) (Ramanzin et al. 2010).

Based on the high nutritional value and low levels of heavy metal accumulation, Sevillano-Morales et al. (2023) suggested that consumption of deer meat needs to be stimulated and become more affordable to a larger number of consumers.

The aim of this study was to collect and analyse relevant scientific data concerning the accumulation of Hg, As, and Cu in the muscles, liver, and kidneys of Roe, Red, and Fallow deer, and to discuss bioaccumulation patterns and the potential risk they pose to consumer health.

Materials and Methods

This narrative review was based on a structured search of the scientific literature to identify and analyse studies published between 2000 and 2024 reporting concentrations of Cu, Hg, and As in the muscles, liver, and kidneys of Roe deer, Red deer, and Fallow deer.

The literature search was conducted using major databases: Scopus, Web of Science, and Google Scholar. The search keywords included combinations such as: 'heavy metal deer', 'deer accumulation heavy metals', 'mercury deer accumulation', 'arsenic deer accumulation', 'copper deer accumulation', etc.

Eligible studies were peer-reviewed articles that provided original research data on the concentrations of one or more of the target metals in at least one of the three specified organs in any of the three deer species in Europe. Studies without specific numerical data, or those analysing only non-edible tissues (e.g., antlers, hair), were excluded. No restrictions were placed on the analytical methods used; however, these were considered when comparing the results.

In total, 10 articles were included for Hg (7 for Roe deer, 4 for Red deer, and 1 for Fallow deer), 9 for As (5 for Roe deer, 4 for Red deer, and none for Fallow deer), and 24 for Cu (7 for Roe deer, 18 for Red deer, and none for Fallow deer). Data were organised according to species, geographical location, organ, detection method, and other variables (sex and age). However, due to the heterogeneity in reporting and missing information in many articles, the data were summarised narratively, and a formal meta-analysis was not conducted.

Results and Discussion

Pathways for heavy metal accumulation

Heavy metals, including toxic elements such as Hg and As, are natural components of Earth's crust and can enter food chains through both geogenic and anthropogenic sources (Tchounwou et al. 2012). Their elevated concentrations in the environment are mainly the result of anthropogenic factors, with industrial development leading to a strong increase in the levels of these pollutants in the air, water, and soil (Durkalec et al. 2015). Game meat can be contaminated with metals and metalloids when animals inhabit anthropogenically

contaminated areas or secondary contamination from hunting ammunition (Taggart et al. 2011).

Table 1 summarises the main sources that can cause the release and accumulation of the investigated heavy metals in the environment.

It is noticeable that the contamination can originate from both natural and anthropogenic sources.

In Europe, the average consumption of ungulate meat is 0.1–0.3 kg per person per year, but in some regions where hunting is popular, the amount can be as high as 1.0–4.0 kg per person per year (Ramanzin et al. 2010). For hunters and their families, the accumulation of heavy metals in meat may pose a risk if the concentrations are high and the intake is above the European average (Lehel et al. 2016).

The main routes of contamination of game meat with heavy metals include the following:

1. Direct intake of contaminated water and soil (mainly by herbivorous mammals) (Beyer et al. 2007, Vardhan et al. 2019).

2. Absorption of heavy metals from contaminated soil and water by plants or deposition of dust and soil particles on the plant surface. Contaminants are thus transported up the food chain; herbivores consume contaminated plants, and predators feed on other contaminated animals. Thus, chain contamination can lead to the accumulation of high metal concentrations in the upper levels of the food chain (Gnamuš et al. 2000, Reglero et al. 2008, Melgar et al. 2009).

3. Inhalation of airborne dust and soil particles containing heavy metals is particularly likely in areas contaminated by mining activities or industrial production (Gerofke et al. 2019, Ma et al. 2020).

4. Cumulative effects on body. Heavy metals are not readily degraded in the body, accumulating over time in various

Table 1. Sources of pollution leading to the accumulation of Hg, As, and Cu in the environment.

Source of pollution	Mercury (Hg)	Arsenic (As)	Copper (Cu)
Mining and smelting activity	✓	✓	✓
Batteries	✓		
Pesticides (general)	✓	✓	✓
Dental procedures	✓		
Traditional medicine	✓		
Fluorescent lamps	✓		
Metal and cement production	✓		✓
Cosmetics	✓		
Volcanic activity	✓	✓	
Hydroelectric systems		✓	
Cigarette smoke		✓	
Transport			✓
Electrical engineering			✓
Energetics			✓
Wastewater irrigation			✓

Note: Adapted from Masindi and Muedi (2018), Izydorczyk et al. (2021), Patel et al. (2023), and Jomova et al. (2025).

tissues and organs: Pb mainly in the bone tissue, Cd in the renal cortex and liver, and methylmercury compounds in the nervous tissue (Rudy 2009). This makes them constantly present in the bodies of animals and increases the risk of increasing their concentration in meat.

Importance of heavy metals for human and animal health

Game meat often accumulates heavy metals because of environmental pollution and human activity. These contaminants can enter the food chain through the soil, water, and animal feed. Subsequently, the consumption of contaminated food can have adverse health effects on consumers (Gerofke et al. 2019, Ciobanu et al. 2020, Nkosi et al. 2021).

Consumption of game meat containing high levels of heavy metals can lead to various health problems in humans, resulting in toxic effects on various organs and systems of the human body.

1. Mercury

Mercury poisoning can cause a variety of toxic effects, depending on its chemical form and route of exposure. Methylmercury, a particularly toxic form of mercury, can cause nervous system damage, loss of coordination and tremors, memory loss, and cognitive and behavioural disorders (Branco et al. 2021). The primary route of human exposure to methylmercury (MeHg) is largely through the consumption of contaminated fish, seafood, and wildlife that have been exposed to mercury through ingestion of contaminated lower organisms. The release of treated mercury can lead to a progressive increase in the amount of atmospheric mercury entering the atmosphere-soil-wa-

ter cycles of distribution, where it can remain in circulation for years (Rice et al. 2014). Mercury poisoning is of particular concern in terms of foetal development, where neurotoxic effects are much more severe than in adults and the threshold for toxicity is lower (Branco et al. 2021). Mercury is hazardous to the developing brain of fetuses and young children and can cause birth defects and developmental delays. Additionally, it can cross the trans-placental barrier and affect foetal growth, leading to premature birth, abortion, and reduced birth weight (Molina-Mesa et al. 2022, Zhang et al. 2023, Wu et al. 2024). High levels of Hg cause oxidative stress, mitochondrial damage, DNA damage, cell membrane damage, etc. (Wu et al. 2024), which is expressed by negative effects on different organs and body systems. Hg accumulation can cause adverse effects and damage the cardiovascular, hematopoietic, immune, respiratory, digestive, excretory, endocrine, and reproductive systems (Rice et al. 2014, Wu et al. 2024, Jomova et al. 2025).

2. Arsenic

Although arsenic occurs naturally in the environment, its concentration can increase as a result of various human activities. As affects multiple biological systems, sometimes years or decades after the exposure is reduced. It can impair pre- and postnatal development in children and cause damage to the skin and nervous, respiratory, cardiovascular, excretory, immune, and endocrine systems (Naujokas et al. 2013). In addition to acute and chronic toxicity, arsenic is a well-known carcinogen that can affect the skin, lungs, gastrointestinal tract, bladder, liver, and kidneys (Hughes 2002). Importantly, this heavy metal can accumulate in

milk, with the highest arsenic levels found in colostrum (Chao et al. 2014), and it was found in the umbilical cord serum and tissue samples from placenta (Jagodić et al. 2022, Molina-Mesa et al. 2022).

3. Copper

Cu is a vital trace element for both humans and animals that serves as an important catalytic cofactor in numerous enzymatic processes, contributing to erythrocyte formation, cellular respiration, energy metabolism, hormone synthesis, collagen production, and cellular defence against oxidative stress, all of which are important for maintaining immunity, growth, and reproductive function (Clarkson et al. 2020, López-Alonso and Miranda 2020).

Nevertheless, ruminants have a narrow tolerance range for Cu and are prone to hepatic accumulation due to complex interactions between Cu, Mo, and sulphur in the rumen, and limited biliary excretion (López-Alonso and Miranda 2020). When Cu intake exceeds storage capacity, animals may develop chronic toxicity, which can lead to liver damage and haemolysis (López-Alonso and Miranda 2020).

Excess free Cu ions can cause damage to cellular components through oxidative stress, DNA damage, and reduced cell proliferation (Gaetke and Chow 2003). They trigger cell death and autophagy via oxidative stress-induced mitochondrial damage, and target lipoylated tricarboxylic acid cycle proteins causing cuproptosis (Wang et al. 2024).

From a food safety perspective, this raises concerns regarding elevated copper accumulation in edible tissues, especially in animals raised in contaminated areas or under copper supplementation. While copper is also essential for human health, frequent consumption of such offal

may pose health risks to sensitive groups such as children and individuals with impaired copper metabolism (López-Alonso and Miranda 2020). High levels of Cu can lead to gastrointestinal problems, vomiting, diarrhoea, and, in extreme cases, liver and kidney damage. Similar to As, Cu can pass through the placental barrier and can be detected in the umbilical cord serum and placental tissue (Jagodić et al. 2022). All these considerations highlight the importance of monitoring copper levels in edible organs to ensure consumer safety.

European regulations: permissible limits for Hg, As, and Cu

To date, there are no regulations on the maximum levels of any heavy metals accumulated in deer meat and edible offal. Notably, the European Commission Regulation 2023/915 (2023) sets limits for Hg in fishery products and bivalve molluscs which range from $0.1 \text{ mg}\cdot\text{kg}^{-1}$ in food supplements to $1.0 \text{ mg}\cdot\text{kg}^{-1}$ in the muscle meat in some fish. However, there is a lack of explicit mention of game or domesticated animal meat, thus creating a regulatory gap. Moreover, there are no regulations for the maximum levels of arsenic and copper in meat or edible offal from any type of animal.

On the other hand, to protect consumer health, FAO, in collaboration with WHO, developed standards for contaminants in food (Codex Alimentarius Commission 2024), according to which the permissible total weekly intake (PTWI) for inorganic mercury is $4 \text{ }\mu\text{g}\cdot\text{kg}^{-1}$ of body weight (bw) for any food other than fish and shellfish, where the intake can reach up to $5 \text{ }\mu\text{g}\cdot\text{kg}^{-1}$ bw. However, the PTWI for methylmercury is set to $0.0016 \text{ mg}\cdot\text{kg}^{-1}$ bw. As for arsenic, the previously accepted PTWI

of $15 \mu\text{g}\cdot\text{kg}^{-1}$ bw for food and water is no longer considered safe and appropriate, and this will be discussed in the future (Codex Alimentarius Commission 2024).

The maximum tolerable daily intake for copper is considered $0.5 \text{ mg}\cdot\text{kg}^{-1}$ bw, according to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (n.d.).

Nonetheless, regular monitoring and research are essential for establishing safe consumption guidelines for wild game, as observed concentrations in different regions may vary due to environmental factors.

Heavy metals accumulations in organs of Roe, Red or Fallow deer

For our analysis, we classified the areas as 'Polluted', 'Unpolluted', or 'n.a.'. The classification was based on the authors' designation (where available) and/or reported proximity to various potential sources of pollution.

1. Mercury

A total of 29 data records from 10 scientific studies reporting Hg concentrations in the muscle, liver, and kidneys of the three cervid species studied were compiled and summarised. Data were obtained from studies across several European countries, including Poland, Hungary, Slovenia, Croatia, and Serbia, some of which include industrial and/or unpolluted regions. The number of animals used in different studies ranged from 20 to 226. Most records do not account for the age and/or sex of animals. The detection methods included AMA-254 (direct mercury analyser), AAS (atomic absorption spectroscopy), ICP-OES (inductively coupled plasma optical emission spectrometry), and ICP-

MS (inductively coupled plasma mass spectrometry).

A summary of the collected data on Hg accumulation in the muscle, liver, and kidney tissues of Roe, Red, and Fallow deer is provided in Table 2.

• Roe deer

Durkalec et al. (2015) compare the accumulation of heavy metals in skeletal muscles, liver and kidneys of Roe deer from polluted and unpolluted areas, and they found that in polluted areas (PA) the highest level of Hg is detected in kidneys, followed by liver, and the lowest level was in the muscles. Similar results were found in unpolluted areas (UPA) (Durkalec et al. 2015). This was the only study with detailed information regarding the levels of this metal in all three organs and in polluted and unpolluted areas. In research conducted by Lehel et al. (2016) on animals from PA was found higher levels of Hg in the musculature of male Roe deer ($1.07 \pm 0.30 \text{ mg}\cdot\text{kg}^{-1}$ w.w.) in comparison to female ($0.61 \pm 0.38 \text{ mg}\cdot\text{kg}^{-1}$ w.w.). Unfortunately, they did not measure Hg levels in the liver or kidneys. Research conducted by Baloš et al. (2015) in UPA and another one by Lehel et al. (2017) in PA did not find traces of Hg in the muscles, liver, or kidneys of Roe deer due to values under the limit of detection of the analytical technique used in their research.

Pokorny and Ribarič-Lasnik (2002) found seasonal differences in Hg levels in Roe deer kidneys. They registered higher levels in late summer and early autumn, which they attributed to seasonal dietary changes, specifically, the increased consumption of mushrooms, which are prone to accumulate various environmental pollutants, including heavy metals (Qin et al. 2024).

Species	Ani- mals, n	Sex	Location	Pollu- tion	Detc- tion method	LODs, mg·kg ⁻¹	Muscle, mg·kg ⁻¹ w.w.	Liver, mg·kg ⁻¹ w.w.	Kidney, mg·kg ⁻¹ w.w.	Source
Roe deer	114	All	Slovenia – Šalek Val- ley (Oct.–Dec.)	PA	AAS	0.010	n.a.	n.a.	0.07 ±0.03 (SE)	Pokorny and Ribarič-Lasnik 2002
	29	All	Slovenia – Pokljuka plateau (May–July)	UPA	AAS	0.010	n.a.	n.a.	0.10 ±0.08 (SE)	Pokorny and Ribarič-Lasnik 2002
	29	All	Slovenia – Pokljuka plateau (Aug.–Sept.)	UPA	AAS	0.010	n.a.	n.a.	0.20 ±0.15 (SE)	Pokorny and Ribarič-Lasnik 2002
	29	All	Slovenia – Pokljuka plateau (Oct.–Dec.)	UPA	AAS	0.010	n.a.	n.a.	0.42 ±0.13 (SE)	Pokorny and Ribarič-Lasnik 2002
	21	All	Slovenia – Kočevski Rog (May–July)	UPA	AAS	0.010	n.a.	n.a.	0.13 ±0.12 (SE)	Pokorny and Ribarič-Lasnik 2002
	21	All	Slovenia – Kočevski Rog (Aug.–Sept.)	UPA	AAS	0.010	n.a.	n.a.	0.45 ±0.33 (SE)	Pokorny and Ribarič-Lasnik 2002
	21	All	Slovenia – Kočevski Rog (Oct.–Dec.)	UPA	AAS	0.010	n.a.	n.a.	0.15 ±0.93 (SE)	Pokorny and Ribarič-Lasnik 2002
	75	All	Slovenia – Šalek Val- ley	PA	AAS	0.010	n.a.	n.a.	0.02 ±0.01 (SE)	Pokorny 2000
	41	All	Slovenia – Koroška region	PA	AAS	0.010	n.a.	n.a.	0.04 ±0.01 (SE)	Pokorny 2000
	28	All	Slovenia – Zasavje region	PA	AAS	0.010	n.a.	n.a.	0.03 ±0.01 (SE)	Pokorny 2000
	20	All	Slovenia – Pokljuka Plateau	UPA	AAS	0.010	n.a.	n.a.	0.23 ±0.10 (SE)	Pokorny 2000

Species	Animals, n	Location	Pollution	Detection method	LODs, mg·kg ⁻¹	Muscle, mg·kg ⁻¹ w.w.	Liver, mg·kg ⁻¹ w.w.	Kidney, mg·kg ⁻¹ w.w.	Source
Red deer	181	Poland	n.a.	AAS	0.001*	0.9 ±1.1	3.2 ±4.9	n.a.	Nawrocka et al. 2020
	14	Poland – Mazury	UPA	AAS	0.001	0.001 ±0.000	0.007 ±0.014	0.048 ±0.102	Gizejewska et al. 2017
	57	Croatia – Baranja	UPA	AAS	n.a.	n.a.	n.a.	0.375 ±0.174	Lazarus et al. 2005
	n.a.	Croatia – Baranja	UPA	AMA-254	n.a.	0.006 ±0.001 (SE)	0.009 ±0.001 (SE)	0.030 ±0.002 (SE)	Lazarus et al. 2008
Fallow deer	44	Poland	n.a.	AAS	0.001*	0.9 ±1.6	2.5 ±4.2	n.a.	Nawrocka et al. 2020

Note: All concentration values are presented as Mean ±SD on a wet weight (w.w.) basis, unless otherwise specified. LOD – limit of detection; * – limit of quantification (LOQ); SE – standard error; PA – polluted area; UPA – unpolluted area.

In a study conducted in PA and UPA of Slovenia, higher levels of Hg were found in the kidneys of animals from the UPA, which the authors attributed to factors outside the sampling habitat, since there was not a single known source of pollution in the UPA (Pokorny 2000).

Nawrocka et al. (2020), in a study from Poland covering the period 2009–2018, found lower accumulation of Hg in muscles ($1.0 \pm 2.1 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$) in comparison to liver ($3.2 \pm 5.7 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$) in Roe deer.

• Red deer

As for the accumulation of Hg in Red deer, a study from Croatia conducted between 2002 and 2003 in UPA revealed $0.375 \pm 0.174 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$ in the kidneys (Lazarus et al. 2005), while another study in the same region found that its accumulation is higher in the kidneys ($0.030 \pm 0.002 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$), followed by the liver ($0.009 \pm 0.001 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$), and the smallest amount was found in the muscles ($0.006 \pm 0.001 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$) (Lazarus et al. 2008). There were relatively large differences between the amount of Hg accumulated in the kidneys in the two studies, which can be attributed to various factors, such as the different limits of detection of the methods used for evaluation its accumulation in both studies, as well as the larger sample size and longer evaluation period in 2008 study (Lazarus et al. 2005, 2008).

The result for higher accumulation of Hg in the kidneys was also confirmed in a study conducted in Poland in UPA. Giżewska et al. (2017) found levels of $0.048 \pm 0.102 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$ in kidneys, $0.007 \pm 0.014 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$ in liver, and $0.001 \pm 0.000 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$ in muscles.

Similar to the results obtained for Roe

deer, Nawrocka et al. (2020) found lower levels of Hg in muscles of Red deer ($0.9 \pm 1.1 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$) compared to liver ($3.2 \pm 4.9 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$).

• Fallow deer

Following the trend of lower Hg concentrations in muscle tissue from Roe and Red deer, within the same study Nawrocka et al. (2020) detected $0.9 \pm 1.6 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$ in muscles and respectively $2.5 \pm 4.2 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$ in liver from Fallow deer. This was actually the only paper concerning the accumulation of Hg in the muscles and liver of Fallow deer, which provides a lot of space for further research on this topic.

• Summary

In most studies, the focus was on Roe deer. Consistent with known toxicokinetics of Hg, its kidney levels in both Roe and Red deer were consistently higher, followed by those in the liver and muscle tissue. Data for Fallow deer were limited but showed elevated liver Hg concentrations compared to muscles.

Although there are no settled limits for Hg in game meat, the European Commission's maximum permissible level in muscle meat from certain fish species is up to $1.0 \text{ mg} \cdot \text{kg}^{-1}$ (Commission Regulation (EU) 2023/915 2023). From the articles covered from 2000 to 2024, only two reported Hg concentrations in meat slightly-exceeding this threshold (Lehel et al. 2016, Nawrocka et al. 2020). In all the other cases, the levels were below it, suggesting a limited risk from muscle meat consumption in most areas. In addition, some liver values in Roe, Red, and Fallow deer exceeded the safety thresholds relevant to human consumption (Nawrocka et al. 2020). The

higher values found in offal emphasize the necessity for targeted monitoring, where game meat contributes substantially to human diets, particularly in industrial regions with high pollution risk.

2. Arsenic

A total of 22 records from nine studies reported As concentrations in one or more of the three organs of interest – muscle, liver, and kidneys – in cervids. Data were obtained from several European countries, including both polluted and/or unpolluted regions. The number of animals used in different studies ranged from 13 to 164. Most records do not account for the age and sex of animals. The detection methods used were AAS, ICP-OES, and ICP-MS. The papers covered As accumulation only in Roe and Red deer, and there was no paper for Fallow deer.

A summary of the collected data on As accumulation in the muscle, liver, and kidney tissues from Roe, Red, and Fallow deer is provided in Table 3.

• Roe deer

In Roe deer, muscle and liver As levels were higher in PA than in UPA, suggesting environmental exposure due to pollution (Baloš et al. 2015, Lehel et al. 2016, Pilarczyk et al. 2020). These results, however, cannot be conclusive because none of the studies made simultaneous comparisons between the accumulation of As in the muscles and liver of animals coming from PA and UPA.

In another research from Slovenia, the levels of As in kidneys in animals originating from both polluted and unpolluted regions did not show significant difference, ranging from 0.14 ± 0.01 to 0.23 ± 0.03 mg·kg⁻¹ w.w. in PA and 0.20

± 0.01 mg·kg⁻¹ w.w. in UPA (Pokorny 2000).

The accumulation of As was found little higher in muscles (0.058 ± 0.013 mg·kg⁻¹ w.w.) than in the liver (0.0507 ± 0.004 mg·kg⁻¹ w.w.) and kidneys (0.042 ± 0.009 mg·kg⁻¹ w.w.) in samples originating from PA (Pilarczyk et al. 2020). This pattern was not observed in samples from UPA, where the levels of As in the different organs were relatively similar (0.014 ± 0.004 mg·kg⁻¹ for muscles; 0.008 ± 0.006 mg·kg⁻¹ for liver and 0.014 ± 0.008 mg·kg⁻¹ for kidneys) (Baloš et al. 2015). It should be noted that the study by Baloš et al. (2015) does not explicitly state whether the values are expressed on a wet or dry weight basis, which may limit comparability with other data.

The presence of mining or industrial activity near a habitat does not always lead to heavy metal accumulation in game animals that reside there. In a study from Hungary, Lehel et al. (2017) did not find detectable amounts of As in any of the three organs of samples obtained from PA.

Although limited, data suggest sex-related variations in As accumulation in Roe deer muscle tissue. Males exhibited a higher mean As concentration (0.33 mg·kg⁻¹ w.w.) compared to females (0.23 mg·kg⁻¹ w.w.), representing approximately 43.5 % increase (Lehel et al. 2016).

• Red deer

In Red deer, only one study from Spain compared the levels of As in muscle tissue from animals originating from the PA and UPA (Taggart et al. 2011). They found that the accumulation of As was higher in PA than in UPA; in the latter, the levels were below the limit of detection of the method for measurement, which was AAS.

Data from samples collected in Spain

Table 3. Reported concentrations of arsenic (As) in muscle, liver, and kidney tissues of Roe deer, Red deer, and Fallow deer from various European regions (2000–2024).

Species	Animals, n	Sex	Location	Pollution	Detection method	LODs, mg·kg ⁻¹	Muscle, mg·kg ⁻¹ w.w.	Liver, mg·kg ⁻¹ w.w.	Kidney, mg·kg ⁻¹ w.w.	Source
Roe deer	20	All	Hungary – Eger	PA	ICP-OES	0.02	0.27 ±0.20	n.a.	n.a.	Lehel et al. 2016
	10	M	Hungary – Eger	PA	ICP-OES	0.02	0.33 ±0.18	n.a.	n.a.	Lehel et al. 2016
	10	F	Hungary – Eger	PA	ICP-OES	0.02	0.23 ±0.23	n.a.	n.a.	Lehel et al. 2016
	11	All	Serbia – Begeč	UPA	ICP-MS	0.001	0.014 ±0.004 (SE)	0.008 ±0.006 (SE)	0.014 ±0.008 (SE)	Baloš et al. 2015
	20	All	Hungary – Pest county	PA	ICP-OES	0.5	<LOD	<LOD	<LOD	Lehel et al. 2017
	10	M	Hungary – Pest county	PA	ICP-OES	0.5	<LOD	<LOD	<LOD	Lehel et al. 2017
	10	F	Hungary – Pest county	PA	ICP-OES	0.5	<LOD	<LOD	<LOD	Lehel et al. 2017
	10	All	Ukraine – Lviv	PA	ICP-OES	1.0 (µg·L ⁻¹)	0.058 ±0.013 (SE)	0.0507 ±0.004 (SE)	0.042 ±0.009 (SE)	Pilarczyk et al. 2020
	75	All	Slovenia – Šalek Valley	PA	AAS	0.010	n.a.	n.a.	0.14 ±0.01 (SE)	Pokorny 2000
	41	All	Slovenia – Koroška region	PA	AAS	0.010	n.a.	n.a.	0.23 ±0.03 (SE)	Pokorny 2000
	28	All	Slovenia – Zasavje region	PA	AAS	0.010	n.a.	n.a.	0.20 ±0.03 (SE)	Pokorny 2000
	20	All	Slovenia – Pokljuka Plateau	UPA	AAS	0.010	n.a.	n.a.	0.20 ±0.01 (SE)	Pokorny 2000

Species	Animals, n	Sex	Location	Pollution	Detection method	LODs, mg·kg ⁻¹	Muscle, mg·kg ⁻¹ w.w.	Liver mg·kg ⁻¹ w.w.	Kidney mg·kg ⁻¹ w.w.	Source
Red deer	13	M	Portugal – Idanha-a-Nova and Lousã	n.a	ICP-MS	0.025	n.a.	0.044 ±0.013 (d.w.)	0.097 ±0.036 (d.w.)	Jota Baptista et al. 2024
	9	M	Portugal – Idanha-a-Nova	PA	ICP-MS	0.025	n.a.	0.046 ±0.014 (d.w.)	0.082 ±0.028 (d.w.)	Jota Baptista et al. 2024
	4	M	Portugal – Lousã	UPA	ICP-MS	0.025	n.a.	0.040 ±0.007 (d.w.)	0.133 ±0.024 (d.w.)	Jota Baptista et al. 2024
	14	All	Poland – Mazury	UPA	AAS	0.002	0.002 ±0.002	0.002 ±0.001	0.003 ±0.002	Gizejewska et al. 2017
	51	All	Spain – Sierra Madrona Mountains and Alcludia Valley	PA	AAS	0.044	0.041 (d.w.)	n.a.	n.a.	Taggart et al. 2011
	37	All	Spain – Montes de Ciudad Real	UPA	AAS	0.044	< LOD	n.a.	n.a.	Taggart et al. 2011
	12	All	Spain – Minas de Horcajo	PA	AAS	0.022	n.a.	0.061 ±0.003 (SE) (d.w.)	n.a.	Reglero et al. 2008
	17	All	Spain – Navalmar-tina	PA	AAS	0.022	n.a.	0.046 ±0.006 (SE) (d.w.)	n.a.	Reglero et al. 2008
	18	All	Spain – Hornias Bajas	UPA	AAS	0.022	n.a.	0.044 ±0.006 (SE) (d.w.)	n.a.	Reglero et al. 2008
	20	All	Spain – Torneros	UPA	AAS	0.022	n.a.	0.047 ±0.007 (SE) (d.w.)	n.a.	Reglero et al. 2008

Note: All concentration values are presented as Mean ±SD on a wet weight (w.w.) basis, unless otherwise specified. LOD – limit of detection; SE – standard error; PA – polluted area; UPA – unpolluted area.

and Portugal showed a higher accumulation of As in the liver of animals coming from PA compared to UPA (Reglero et al. 2008, Jota Baptista et al. 2024). Data from Portugal also show higher levels of As accumulation in kidneys in UPA than in PA; however, the number of animals tested from UPA was very low, which makes it difficult to draw a general conclusion.

The only data for the accumulation of As in the muscles, liver, and kidneys were from Poland, and no statistically significant differences were found between the As levels in the different organs (Giżewska et al. 2017).

• Summary

Overall, studies on the accumulation of As in organs from Roe deer, used for human consumption, indicated that the levels are relatively low even in polluted areas. However, since the maximum permissible concentrations of this metal in meat and offal are not set by the FAO/WHO (Codex Alimentarius Commission 2024), there is still a risk that the consumption of contaminated game meat or organs can contribute to chronic exposure in regular consumers, especially in industrial or agricultural regions.

The insufficient data on the accumulation of As in Roe and Red deer and the absence of up-to-date data in Fallow deer show a gap that needs to be filled in the future.

3. Copper

A total of 41 records from 24 studies that reported Cu concentrations in deer tissues were analysed. The dataset included information on Roe and Red deer, with Red deer being the most studied species. No data were available for Fallow deer

from 2000 to 2024. Data came from several European countries, including Bulgaria, Croatia, Poland, Portugal, Scotland, Spain, Slovakia, Serbia, and Ukraine, including both polluted and unpolluted regions. The number of animals used in the different studies ranged from 4 to 435. Most studies do not account for the age and/or sex of the animals. The detection methods used were AAS, ICP-OES, and ICP-MS.

A summary of the collected data on Cu accumulation in the muscle, liver, and kidney tissues of Roe, Red, and Fallow deer is provided in Table 4.

• Roe deer

Several studies conducted in Poland found lower Cu accumulation in muscle tissue from Roe deer originating from the UPA compared to those from the PA (Długaszek and Kopczyński 2013, Cygan-Szczegielniak and Stasiak 2022).

In a study by Cygan-Szczegielniak and Stasiak (2022), they found higher accumulation in muscles than in the liver, and female animals had slightly higher levels of Cu in both muscle tissue and in liver than males.

In a study in Serbia, it was found that the liver had the highest accumulation of Cu, followed by the kidneys, and the lowest level was found in the muscles (Baloš et al. 2015). Higher accumulation in the liver than in the kidneys was also observed by Wieczorek-Dąbrowska et al. (2013) in Poland and Ahmed and Markov (2017) in Bulgaria. These findings were partially confirmed by Pilarczyk et al. (2020) in a study from Ukraine, where the liver also had higher Cu accumulation, followed by the muscles and kidneys. Interestingly in another research Markov and Ahmed (2019) registered several times

Table 4. Reported concentrations of copper (Cu) in muscle, liver, and kidney tissues of Roe deer, Red deer, and Fallow deer from various European regions (2000–2024).

Species	Animals, n	Sex	Location	Pollution	Detection method	LODs, mg·kg ⁻¹	Muscle, mg·kg ⁻¹ w.w.	Liver, mg·kg ⁻¹ w.w.	Kidney, mg·kg ⁻¹ w.w.	Source
Roe deer	28	M	Poland – Kuyavian-Pomeranian region	PA	AAS	n.a.	37.3 ±9.91 (d.w.)	19.7 ±5.89 (d.w.)	n.a.	Cygan-Szczegielniak and Stasiak 2022
	28	F	Poland – Kuyavian-Pomeranian region	PA	AAS	n.a.	39.5 ±10.5 (d.w.)	20.9 ±6.24 (d.w.)	n.a.	Cygan-Szczegielniak and Stasiak 2022
	11	All	Serbia – Begeč	UPA	ICP-MS	0.001	4.2305 ±0.9411 (SE)	28.071 ±34.026 (SE)	15.871 ±6.698 (SE)	Baloš et al. 2015
	10	All	Ukraine – Lviv	PA	ICP-OES	0.4 (µg·L ⁻¹)	2.20 ±0.236 (SE)	10.4 ±2.65 (SE)	0.840 ±0.821 (SE)	Pilarczyk et al. 2020
	25	M	Poland – Zachodniopomorskie province	PA	ICP-OES	0.00296	n.a.	4.052 ±0.389 (SE)	3.770 ±0.353 (SE)	Wieczorek-Dąbrowska et al. 2013
	13	All	Poland – Lodz and Mazovia Voivodships	UPA	AAS	n.a.	1.65 ±1.24	n.a.	n.a.	Długaszek and Kopczyński 2013
Red deer	10	M	Bulgaria – West Stara Planina	PA	ICP-OES	n.a.	n.a.	16.2 ±3.5 (d.w.)	52.7 ±6.2 (d.w.)	Markov and Ahmed 2019
	15	M	Bulgaria – East	n.a.	AAS	n.a.	n.a.	65.431 ±89.3 (d.w.)	27.948 ±33.6 (d.w.)	Ahmed and Markov 2017
	20	M	Poland – Kuyavian-Pomeranian province	PA	AAS	n.a.	43.41 ±0.418 (SE) (d.w.)	38.42 ±0.194 (SE) (d.w.)	16.70 ±0.190 (SE) (d.w.)	Cygan-Szczegielniak 2021
	13	M	Portugal – Idanha-a-Nova and Lousã	n.a	ICP-MS	0.01	n.a.	132.141 ±39.028 (d.w.)	24.514 ±4.639 (d.w.)	Jota Baptista et al. 2024
	9	M	Portugal – Idanha-a-Nova	PA	ICP-MS	0.01	n.a.	150.059 ±33.321 (d.w.)	25.439 ±5.019 (d.w.)	Jota Baptista et al. 2024

Species	Animals, n	Sex	Location	Pollution	Detection method	LODs, mg·kg ⁻¹	Muscle, mg·kg ⁻¹ w.w.	Liver, mg·kg ⁻¹ w.w.	Kidney, mg·kg ⁻¹ w.w.	Source
Red deer	4	M	Portugal – Lousã	UPA	ICP-MS	0.01	n.a.	91.828 ±1.319 (d.w.)	22.432 ±3.250 (d.w.)	Jota Baptista et al. 2024
	10	All	Poland – Warmia and Mazury	UPA	AAS	n.a.	n.a.	27.64 ±9.51	n.a.	Cebulska et al. 2021
	13	All	Poland – Zinc Smelter Miasteczko Slaskie	PA	AAS	0.2	n.a.	17.91 (Mean)	4.47 (Mean)	Durkalec et al. 2017
	9	All	Poland – Masurian Lake District	UPA	AAS	0.2	n.a.	1.96 (Mean)	8.59 (Mean)	Durkalec et al. 2017
	50	F	Poland – Lower Silesia and Warmia and Mazury	n.a.	ICP-MS	n.a.	2.79 ±0.55	n.a.	n.a.	Skibniewski et al. 2015
	23	F	Poland – Lower Silesia	PA	ICP-MS	n.a.	2.99±0.48	n.a.	n.a.	Skibniewski et al. 2015
	27	F	Poland – Warmia and Mazury	UPA	ICP-MS	n.a.	2.61 ±0.54	n.a.	n.a.	Skibniewski et al. 2015
	14	All	Poland – Warmia and Mazury	UPA	AAS	0.04	1.10 ±0.35	7.29 ±7.02	4.08 ±0.45	Giżejewska et al. 2017
	82	All	Poland – Warmia and Mazury	UPA	ICP-MS	n.a.	3.3±1.0	16.0±7.0	5.2±1.0	Falandysz et al. 2005
	17	Fawns	Poland – Warmia and Mazury	UPA	ICP-MS	n.a.	3.5±1.0	8.3±2.0	5.1±1.1	Falandysz et al. 2005
	29	F	Poland – Warmia and Mazury	UPA	ICP-MS	n.a.	3.3±1.1	18±6	5.5±1.0	Falandysz et al. 2005
	36	M	Poland – Warmia and Mazury	UPA	ICP-MS	n.a.	3.2±0.9	15±8	5.0±0.8	Falandysz et al. 2005
	20	All	Poland – Warmia and Mazury	UPA	ICP-MS	n.a.	11±4 (d.w.)	59±41 (d.w.)	21±4 (d.w.)	Jarzyńska and Falandysz 2011

Species	Animals, n	Sex	Location	Pollution	Detection method	LODs, mg·kg ⁻¹	Muscle, mg·kg ⁻¹ w.w.	Liver, mg·kg ⁻¹ w.w.	Kidney, mg·kg ⁻¹ w.w.	Source
Red deer	25	M	Poland – Zachodniopomorskie province	PA	ICP-OES	0.00296	n.a.	3.928±0.544 (SE)	2.453±0.315 (SE)	Wieczorek-Dąbrowska et al. 2013
	n.a.	All	Croatia – Baranja	UPA	AAS	n.a.	3.48±0.28 (SE)	14.7±2.1 (SE)	4.51±0.26 (SE)	Lazarus et al. 2008
	57	All	Croatia – Baranja	UPA	AAS	n.a.	n.a.	n.a.	5.16±1.00	Lazarus et al. 2005
	22	M	Slovakia – Topolčany	UPA	AAS	0.015 (mg/L)	4.934 (median)	13.342 (median)	4.934 (median)	Gasparik et al. 2004
	51	All	Spain – Sierra Madrona Mountains and Alcudia Valley	PA	AAS	13.2	10.4 (d.w.)	n.a.	n.a.	Taggart et al. 2011
	37	All	Spain – Montes de Ciudad Real	UPA	AAS	13.2	9.1 (d.w.)	n.a.	n.a.	Taggart et al. 2011
	28	All	Spain – Sierra Madrona Mountains and Alcudia Valley	PA	AAS	0.009	n.a.	75.71 (d.w.)	n.a.	Rodríguez-Estival et al. 2011a
	26	All	Spain – Montes de Ciudad Real	UPA	AAS	0.009	n.a.	67.02 (d.w.)	n.a.	Rodríguez-Estival et al. 2011a
	40	All	Spain – Cordoba	n.a.	AAS	0.078	1.87±0.76	n.a.	n.a.	Sevilano-Morales et al. 2023
	12	All	Spain – Minas de Horcajo	PA	AAS	1.48	n.a.	50.8 ±7.8 (SE) (d.w.)	n.a.	Reglero et al. 2008
	17	All	Spain – Navamartina	PA	AAS	1.48	n.a.	51.7 ±9.1 (SE) (d.w.)	n.a.	Reglero et al. 2008
	18	All	Spain – Hornias Bajas	UPA	AAS	1.48	n.a.	47.3 ±6.4 (SE) (d.w.)	n.a.	Reglero et al. 2008

Species	Animals, <i>n</i>	Sex	Location	Pollution	Detection method	LODs, $\text{mg}\cdot\text{kg}^{-1}$	Muscle, $\text{mg}\cdot\text{kg}^{-1}$ w.w.	Liver, $\text{mg}\cdot\text{kg}^{-1}$ w.w.	Kidney, $\text{mg}\cdot\text{kg}^{-1}$ w.w.	Source
Red deer	20	All	Spain – Torneros	UPA	AAS	1.48	n.a.	55.9 $\pm$ 6.9 (SE) (d.w.)	n.a.	Regjero et al. 2008
	435	M	Scotland	n.a.	ICP-OES	n.a.	n.a.	44.2 (Mean) (d.w.)	n.a.	French et al. 2017
	352	F	Scotland	n.a.	ICP-OES	n.a.	n.a.	66.5 (Mean) (d.w.)	n.a.	French et al. 2017
	n.a.	n.a.	Bulgaria – the Rhodopes Mountains	n.a.	ICP-OES	n.a.	n.a.	76.7 (d.w.)	n.a.	Markov et al. 2020 (AO)
	n.a.	n.a.	Bulgaria – Central Stara Planina	n.a.	ICP-OES	n.a.	n.a.	115.6 (d.w.)	n.a.	Markov et al. 2020 (AO)

Note: All concentration values are presented as Mean $\pm$ SD on a wet weight (w.w.) basis, unless otherwise specified. LOD – limit of detection; SE – standard error; PA – polluted area; UPA – unpolluted area. AO – abstract only.

higher levels of Cu in kidneys (52.7 $\pm$ 6.2 $\text{mg}\cdot\text{kg}^{-1}$ d.w.) than in the liver (16.2 $\pm$ 3.5 $\text{mg}\cdot\text{kg}^{-1}$ d.w.). The authors stated that additional research on the pollution with heavy metals of the environmental factors, locally grown grass and tree species used for grazing by the Roe deer, and externally delivered food is necessary to define the reason for such a large difference in metal accumulation between the two organs.

• Red deer

Red deer accumulate Cu at higher levels than other wild species do. In a study conducted in north-eastern Poland, Cu accumulation in Red deer liver was more than five times higher than that in Red foxes, and more than nine times higher than that in Wild boar (Cebulska et al. 2021). In Cebulska et al. (2021), metal concentrations in the liver tissue of Wild boar, Red fox, and Red deer were presented without specifying whether they refer to wet or dry weight, which makes direct comparisons with other studies difficult.

In most studies where Cu levels were compared among the three organs in Red deer, the highest accumulation was found in the liver, followed by the kidneys and muscles (Falandysz et al. 2005, Jarzyńska and Falandysz 2011, Giżejewska et al. 2017). Similarly, when the accumulation of Cu was tested only in the liver and kidneys, the liver was again shown to have a higher concentration of Cu than the kidneys, consistent with the physiological role of the liver in metal storage and detoxification (Wieczorek-Dąbrowska et al. 2013, Jota Baptista et al. 2024).

Interestingly, in a study from Poland where the accumulation of this heavy

metal was measured in the liver and kidneys of animals originating from PA and UPA, higher levels of Cu were found in the liver of PA, whereas in UPA, the kidneys had higher Cu content (Durkalec et al. 2017).

Exceptions from that trend are the two papers – one from Poland, where the muscles had higher Cu accumulation ($43.41 \pm 0.418 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$) than liver ($38.42 \pm 0.194 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$) and kidneys ($16.70 \pm 0.190 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$) (Cygan-Szczegielniak 2021) and one from Slovakia where the liver had highest median Cu content with $13.342 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$, while muscles and kidneys were equal with median amount of $4.934 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$ each (Gasparik et al. 2004).

Cu levels were generally higher in all organs from PA (Rodríguez-Estival et al. 2011b, Skibniewski et al. 2015, Jota Baptista et al. 2024) with partial exception in the research of Durkalec et al. (2017) where the liver had higher accumulation in PA, however the kidneys had higher accumulation in UPA, and a research from Spain (Reglero et al. 2008), where in one of the two UPA tested the liver accumulation of the metal was little higher ($55.9 \pm 6.9 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$) than any of the two PA included in the research ($50.8 \pm 7.8 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$ and $51.7 \pm 9.1 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$). Taggart et al. (2011) found elevated levels of Cu in muscle from Red deer coming from PA ($10.4 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$) than UPA ($9.1 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$).

Markov et al. (2020), in research conducted in Bulgaria, found considerable difference between the levels of Cu accumulated in the liver of Red deer originating from the Rhodopes Mountains – $176.7 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$ in comparison to $115.6 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$ in animals from Central Stara Planina Mountain. The authors suggested that Red deer can serve as a

bioindicator of the heavy metal pollution of the environment resulted from the anthropogenic activities.

In a study on Red deer from Scotland, higher levels of Cu were found in the liver of female animals, with mean value of $66.5 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$, in comparison to male with mean value of $44.2 \text{ mg} \cdot \text{kg}^{-1} \text{ d.w.}$ (French et al. 2017).

• Summary

Although the liver is often the organ with the highest accumulation of Cu, especially in animals from PA, followed by the kidneys and muscles, some studies have reported findings that differ from this trend. We believe that many factors can contribute to these differences. The various methods of detection with different sensitivities, the source of pollution in the polluted area, the levels of the metal in the environment of the unpolluted area, and the size of the sample are all parameters that can affect the results and must be considered.

The maximum tolerable daily intake for copper is considered to be $0.5 \text{ mg} \cdot \text{kg}^{-1} \text{ bw}$, according to JECFA (n.d.). It can be concluded that consumption of meat, liver and kidney from Roe and Red deer is relatively safe, especially if they come from UPA and consumption is not excessive. Nonetheless, in some cases, mostly from PA, Cu accumulation in the muscles and liver is high, which should be taken into consideration (Cygan-Szczegielniak 2021, Jota Baptista et al. 2024).

Conclusions

The organs with the highest accumulation of Hg are the kidneys, followed by the liver, and to a lesser extent, muscles. The sea-

son of sample collection is important for the final results, and sex also plays a role, as males accumulate more metal than females. In the case of As, accumulation was slightly higher in muscle tissue than in the liver and kidneys. Cu was found to accumulate predominantly in the livers of Roe and Red deer, followed by the kidneys and muscles, with several exceptions. While most of the reviewed studies reported concentrations of Cu, Hg, and As in deer tissues within acceptable limits, isolated cases of elevated levels, particularly in animals from polluted areas, highlight the need for regular monitoring and cautious consumption, especially of the liver and kidneys.

There are very few studies providing simultaneous measurements of the accumulation of any of the metals Hg, As, or Cu in all three organs at once – muscle, liver, and kidneys of Roe deer. Even less data are available on their accumulation in Red and Fallow deer. In the case of Fallow deer, no information on As and Cu accumulation has been reported in the scientific literature over the past 24 years. Another limitation of the studies conducted to date is the lack of comparison between male and female animals, as well as the limited number of studies comparing heavy metal accumulation in animals from polluted and unpolluted areas.

Various detection methods, each with different limitations, were used, which did not allow an exact comparison of the results.

Given these findings, there is an obvious need for more comprehensive research on the topic, which can help develop programmes for regular monitoring of heavy metal accumulation in organs of deer used for human consumption, and possibly defining the permissible levels of Hg, As, and Cu in game meat and offal.

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NDVI monitoring under drip irrigation in semi-natural ecosystems

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Thematic field: Agriculture in mountainous and semi-mountainous regions

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Abstract

The objective of this study was to enhance the analysis of vegetation information using the normalised difference vegetation index (NDVI) through remote sensing data. NDVI values were correlated with ground measurements to derive insights into vegetation health and productivity. The experiment focused on courgette cultivation, where NDVI was measured at full growth using a Trimble GreenSeeker handheld sensor. Various nitrogen and phosphorus fertilisers were applied, including ammonium nitrate, phosphates, and polyphosphates. The highest NDVI value achieved was 0.802 with ammonium nitrate (nitrogen treatment) lacking phosphorus. On a polyphosphate background, the nitrogen source with the highest NDVI was KSC, yielding 0.800. With an orthophosphate background, KSC produced the best NDVI of 0.815. Its highest values were consistently linked with the orthophosphate background, and these results were statistically validated. A strong correlation coefficient ($r = 0.72$) was observed between NDVI and courgette yield. The integration of NDVI into our analytical approach significantly refined the analysis of vegetation data, yielding more precise insights.

Key words: fertigation, managed ecosystems and agroforestry, nitrogen, phosphorus, potassium, remote sensing.

Introduction

Enhancing the analysis of vegetation information using remote sensing data is the main goal of normalised difference vegetation index (NDVI). Research has demonstrated that it is useful for evaluating a range of vegetative characteristics, including leaf area index which is a measurement representing the total one-sided green leaf area per unit of ground area in a vegetation canopy (LAI) (Tian et al. 2017), leaf chlorophyll concentration (Pastor-Guzman et al. 2015), plant productivity (Vicente-Serrano et al. 2016), and plant stress (Chavez et al. 2016). Such estimates are often derived by correlating the NDVI values measured remotely with the ground-measured values of these variables. The resilience of NDVI-related models is directly influenced by NDVI reliability (Butt 2018). Its first application in Bulgaria was in forest observations for bark beetle invasion (Stoyanova et al. 2018).

Additional studies have shown that various spectral vegetation indices, calculated from visible and near-infrared (NIR) reflectance data, are linearly related to canopy light interception (López-Urrea et al. 2009). For several horticultural crops a substantial correlation between fractional canopy cover (F_c) and the Landsat-derived NDVI was demonstrated. Therefore, it seems that indices like NDVI may be able to monitor trees growth and light efficiency interception (Trout et al. 2008).

About 2.0 million hectares of land in the EU were used to produce fresh vegetables in 2022, representing an estimated 1 % of the EU's total utilised agricultural area, on which 59.8 million tonnes of crops were produced. Courgettes (zucchini), from the group of fresh vegetables cultivated for fruits, accounted for more than

one-quarter (27.2 %) of the area. Fruit and vegetable production levels are influenced by weather, just as other crop types (EUROSTAT 2024).

Courgettes belongs to Cucurbitaceae and is used for fresh consumption, processing, and preservation. The fruits are rich in vitamins and mineral salts, fibre, carbohydrates, and pectic substances. Their high biological value is also complemented by the low calorific value of the fruit, 34 kcal per 100 g fresh mass (Balla 1977).

Courgettes is an important market crop for both open-field and protected cultivation in South Europe. For example, in Italy more than 12,000 ha are cultivated each year (ISTAT 2024). Courgettes are cultivated in soil under greenhouse conditions mainly using a drip-irrigation system in order to respond to the high market demand (Rouphael and Colla 2005).

Courgettes prefer good, irrigated conditions (Coleman et al. 2015, Contreras et al. 2017). That is why drip irrigation is indispensable. By improving irrigation efficiency, this system can deliver nutrients and water directly to the crop's root zone, increasing output while conserving water (Santosh et al. 2017). In the conditions of fertigation with nitrogen fertilisers, the highest yield of courgettes was obtained on a phosphorus background composed of orthophosphates (Nikolov et al. 2018).

NDVI, which is calculated by dividing the difference between the red and NIR reflectance by their sum, is one of the most used spectral vegetation indices produced from remotely sensed imagery (Tucker 1980). Previous studies showed that NDVI is strongly related to the fraction of absorbed photosynthetically active radiation (FPAR) (Gallo et al. 1985), leaf area index (LAI) (Wang et al. 2005), and net primary production (NPP) (Potter 1999).

NDVI has also been used in a range of applications, including the study of vegetation-climate interactions (Donohue et al. 2009), detection of long-term vegetation changes (Eklundh and Olsson 2003), assessment of vegetation functional characteristics (Lu et al. 2003), and modelling of the carbon balance at the global level (Fang et al. 2003).

The objective of this study was to enhance the analysis of vegetation information using the normalised difference vegetation index (NDVI) through remote sensing data. These values were correlated with ground-based measurements to extract insights into vegetation health and productivity, particularly in relation to fertiliser application and its effects on crop growth.

Materials and Methods

Normalised difference vegetation index – NDVI

The NDVI formula is based on the principle that chlorophyll strongly absorbs radiation in the red spectrum, while the internal leaf structure reflects radiation in the NIR region. NDVI values therefore, range from -1 to $+1$, where negative values correspond to non-vegetated surfaces (Myeni et al. 1995). The index is calculated using equation (1):

$$NDVI = \frac{NIR - RED}{NIR + RED}, \quad (1)$$

where: NIR – near-infrared reflectance; RED – red reflectance in the visible spectrum.

NDVI is a coefficient that relates the reflection of red and near-infrared light. It measures the amounts of NIR and red light reflected by vegetation, as captured by sensors on satellites, drones, or

ground-based instruments.

NDVI is associated with many plant properties. It is used to determine the health status of plants, to follow phenological changes, green biomass and crop yield estimation, and for other applications. It has been used for decades as a predictor of vegetative biomass (Tucker 1979).

In the conditions of the experiment carried out, the NDVI was measured using a handheld sensor manufactured by N-Tech Industries and marketed by Trimble, the Trimble GreenSeeker (Fig. 1). Measurements were taken after the last fertilization event during sunny midday conditions.



Fig. 1. Trimble GreenSeeker handheld sensor.

Test crop – Courgettes

The experiment was conducted in 2018 at Vrazhdebna Training and Experimental Field Centre, near Sofia, Bulgaria.

In the first year of the research, a field fertiliser experiment with courgettes was set. The variety is Eskenderany F1. This is a hybrid courgette with a light green colour, produced by Seminis. The plants are strong, half-upright, and compact, resistant to low temperatures. The growing season is 40–45 days after transplanting. The fruits are glossy with a length of 18–

20 cm.

The water regime was maintained by drip irrigation according to the developmental stage and requirements of the courgettes. Water content during the growing period was at 80 % of field capacity. Fertilisers were applied with the irrigation, with rates depending on crop growth intensity.

Fertilisers and tools

Fertilisers with a different source of nitrogen (ammonium and nitrate) and phosphorus (orthophosphate and polyphosphate) were used.

Sources of nitrogen

The following fertilisers with varying nitrogen forms were used:

1. KSC III for vegetables – a complex fertiliser containing N – 15 %, P_2O_5 – 5 %, K_2O – 35 %, B – 0.1 %, Fe – 0.1 %, Mo – 0.1 %. Nitrogen is represented by 11.7 % nitrate and 3.3 % ammonium nitrogen.

2. SULFAMMO (N-PRO) contains N – 25 %, SO_3 – 31 %, MgO – 2 %. Based on ammonium sulphate.

3. Ammonium nitrate $[(NH_4)NO_3]$ is an ammonium nitrate fertiliser (N – 34 %).

4. Ammonium sulphate $[(NH_4)_2SO_4]$ – an ammonium-based fertiliser (N – 21 %) – to equalize the treatments with Eurofertil Plus 36 to those with Duofertil TOP 34.

Sources of phosphorus

The phosphorus fertilisers used in the experiment included both polyphosphate- and orthophosphate-based products. Their main characteristics are summarised in Table 1.

Potassium source

Potassium sulphate (K_2SO_4) – 50 % K_2O – to equalise the amounts of potassium.

Most fertilisers contain more than one nutrient. Macro elements are equal in content in each treatment by the means of simple fertilisers. There is no complete alignment of trace elements by treatment. The approach was to carry out trials with complete macronutrient fertilisation and conditions close to practice. Fertilisers with a modern formulation from one manufacturer are widely used by farmers.

Table 1. Phosphorus fertilisers used in the experiment.

Fertiliser (trade name)	Manufacturer, country	Composition, %	Form of phosphorus	Notes
Duofertil TOP 34	Timac Agro, France (via Timac Agro Bulgaria)	N-P-K: 5-19-10; SO_3 : 19; B: 0.1; Zn: 0.1	Polyphos- phates	Main P source
Eurofertil Plus 36 (Physio+)	Timac Agro, France (via Timac Agro Bulgaria)	P_2O_5 : 12; K_2O : 24; SO_3 : 15; B: 0.2	Orthophos- phates	Main P source
Triple superphos- phate	Agropolychim AD, Bulgaria	P_2O_5 : 46	Orthophos- phates	Used to equalise P level with Duofertil TOP 34 treatments

Note: Trade names are mentioned solely for the purpose of providing specific information. Their inclusion does not imply endorsement or recommendation by the authors or the journal.

Results and Discussion

Fertilisers influence on courgette yields is presented in Figure 2. It is obvious that the polyphosphate source is negatively acting on courgette yield. The orthophosphate source of Eurofertil has a strong positive impact on all treatments.

Developmental quality of courgettes was expressed and assessed by NDVI and was determined at full growth. Phosphorus sources have the same impact on NDVI as in the yield survey. On background phosphorus fertilisation, the fertilisers SULFAMMO and KSC performed best. Orthophosphates gave the best basis for plant development, and in them the indicators for all nitrogen fertilisers and control were highest (Fig. 3 and Table 2).

Statistics of obtained results

The results were treated statistically with an ANOVA multifactorial analysis for the influence of nitrogen fertilisers on phosphorus fertilisers as background and for the influence of phosphorus fertilisers on nitrogen fertilisers as background. In the first analysis it is seen that all nitrogen fertilisations differ significantly from the control treatment for NDVI (Fig. 4). KSC fertiliser application results are statistically different than other nitrogen fertilisers, too. The impacts of fertilisers SULFAMMO and ammonium nitrate are not statistically different between them. Phosphorus fertilisation with orthophosphates differed significantly from the control and polyphosphate treatment (Fig. 5). There is no sta-

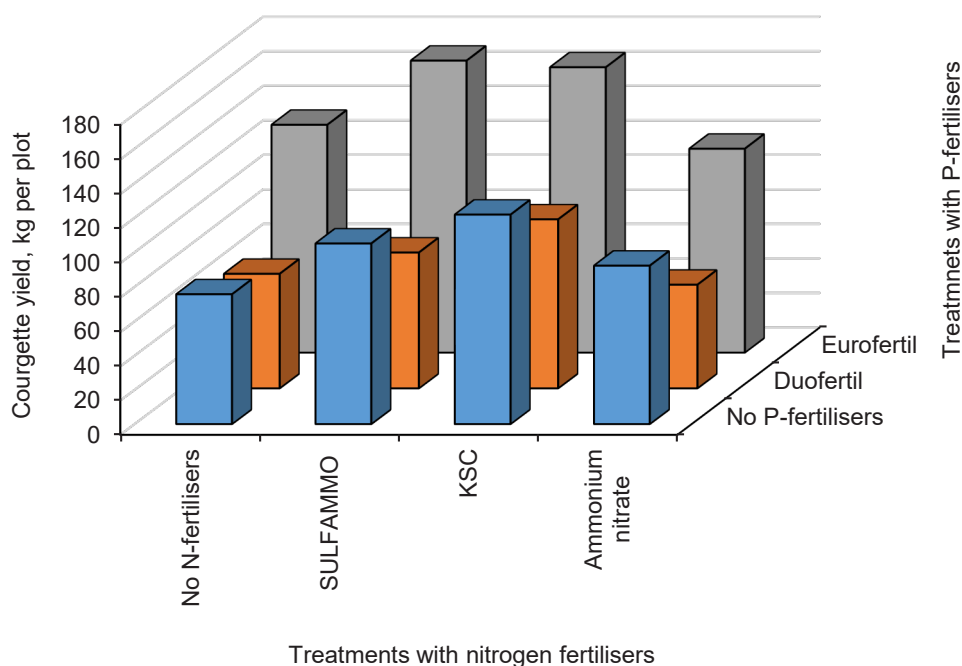


Fig. 2. Courgette yield depending on the fertilisation with nitrogen and phosphorus fertilisers.

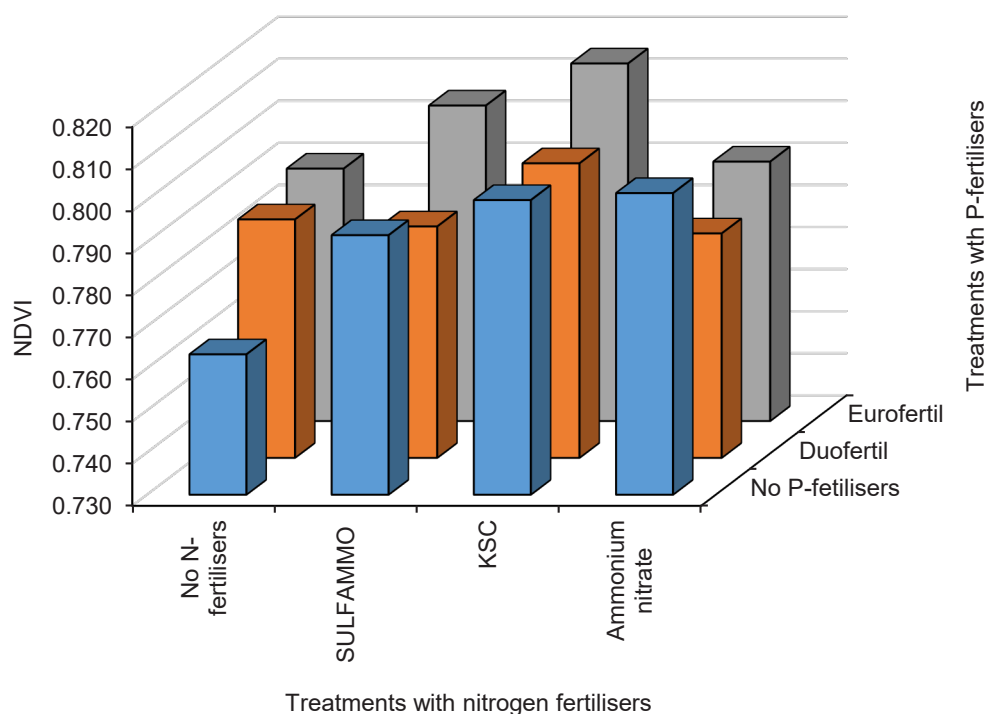


Fig. 3. NDVI depending on the fertilisation of courgettes with nitrogen and phosphorus.

Table 2. NDVI of courgettes under nitrogen and phosphorus fertilisation.

Treatments	No fertilisers	SULFAMMO	KSC	Ammonium nitrate
Control	0.763	0.792	0.800	0.802
Duofertil	0.787	0.785	0.800	0.783
Eurofertil	0.790	0.805	0.815	0.792

tistical evidence that treatments Control and Duofertil are different.

Table 3 presents the Pearson product-moment correlation coefficients between each pair of studied variables in the 0–30 cm soil layer. These coefficients range from -1 to $+1$ and indicate the strength and direction of the linear relationship between the variables.

Correlation coefficients smaller than 0.3 show a weak strength, between 0.3 and 0.7 is a medium strength, and larger than 0.7 shows a strong correlation

strength. A P -value, which assesses the statistical significance of the estimated correlations, is the second figure in each place in the table. P -values below 0.05 indicate statistically significant non-zero correlations at the 95.0 % confidence level. The following pairs of variables have P -values below 0.05: N and Yield; NDVI and Yield. The negative correlation between inorganic nitrogen content and courgette yield is explained by the stronger nitrogen assimilation and uptake in higher-yielding treatments.

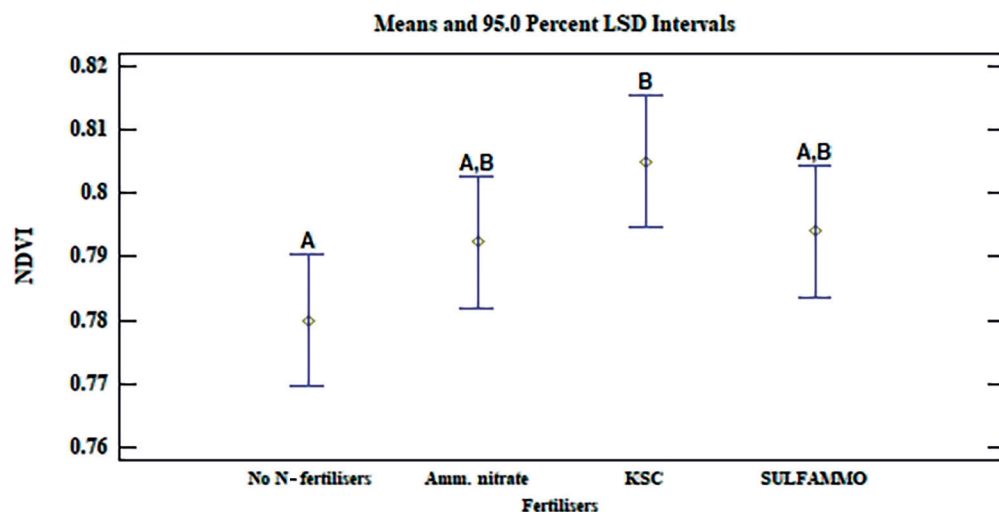


Fig. 4. Graphical presentation of lower and upper limit of N-fertilisers treatments.

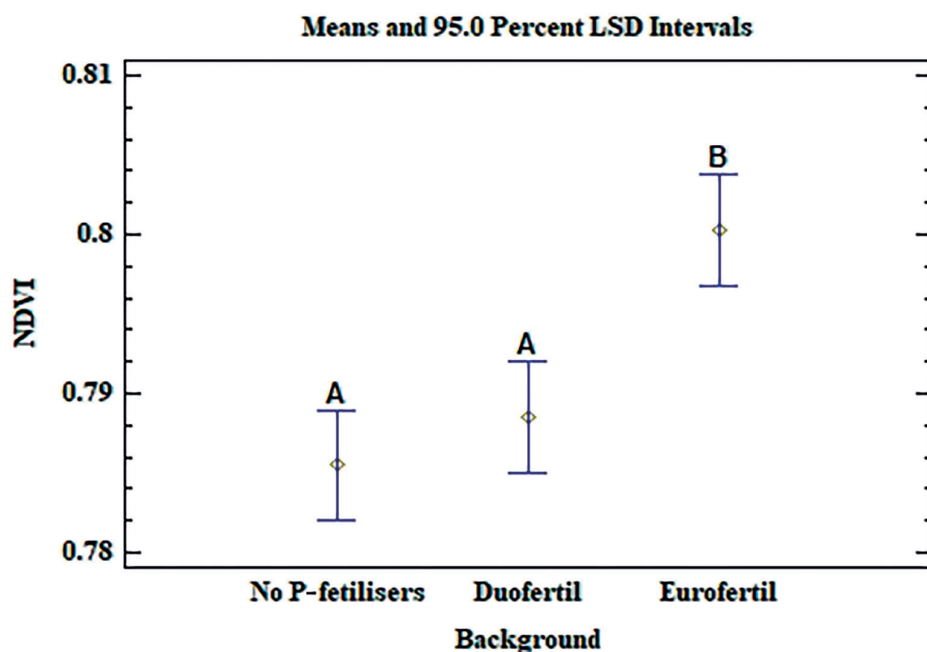


Fig. 5. Graphical presentation of lower and upper limit of P-background treatments.

Simple regression exists between NDVI as an independent variable and Yield as a dependent variable, which is

showing their quantitative relation described by the following linear model – equation (2):

Table 3. Pearson correlation coefficients between the studied indicators in 0–30 cm soil layer.

Variable	EC	K	N	NDVI	P	Yield
EC	1	–0.2579 (0.4184)	–0.3463 (0.2702)	–0.0423 (0.8961)	–0.2757 (0.3857)	0.2984 (0.3461)
K		1	–0.1929 (0.5481)	0.4455 (0.1466)	0.3824 (0.2198)	0.2056 (0.5214)
N			1	–0.5653 (0.0554)	0.3593 (0.2514)	–0.5853 (0.0456)
NDVI				1	–0.0489 (0.8801)	0.7163 (0.0088)
P					1	–0.2632 (0.4085)
Yield						1

Note: EC – electrical conductivity; K – potassium; N – nitrogen; P – phosphorus; NDVI – Normalised Difference Vegetation Index. Values above the diagonal represent Pearson correlation coefficients. Values in parentheses are the corresponding *P*-values.

$$Y = a + b \cdot X. \quad (2)$$

The equation of the fitted model is:

$$\text{Yield} = -1433.36 + 1942.86 \cdot \text{NDVI}. \quad (3)$$

Since the *P*-value in the ANOVA table is 0.0088 which is less than 0.05, there is a statistically significant relationship between Yield and NDVI at the 95.0 % confidence level. The *R*-squared statistic in equation (3) indicates that the model as fitted explains 51.31 % of the variability in yield. The correlation coefficient equals 0.716, indicating a moderately strong relationship between the variables (Fig. 6).

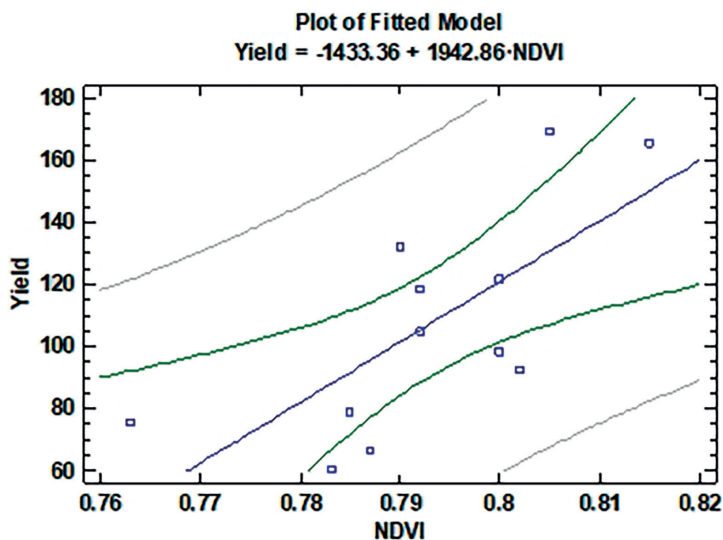
Conclusions

The overall objective of using NDVI to improve the analysis of vegeta-

tion information with remote sensing data is successful.

In experimental conditions, the application of orthophosphates showed better results than polyphosphates.

On a phosphorus background, ammonium was a more effective nitrogen source

**Fig. 6. Plot of fitted model for yield in kg.**

than ammonium nitrate.

Compared to other nitrogen fertilisers, KSC fertilisers have a higher priority in plant nutrition because they supply microelements in addition to nitrogen.

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Influence of forest canopy cover on automated Avalanche Terrain Exposure Scale classification in the Pirin Mountains, Bulgaria

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Thematic field: Forest disturbances and management challenges

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Abstract

Backcountry skiing and touring have gained significant popularity in recent years, with numerous accidents occurring annually worldwide. Bulgaria's Pirin Mountains have experienced similar trends, highlighting the urgent need for tools that enhance public awareness of avalanche-prone terrain. The Avalanche Terrain Exposure Scale (ATES) offers a systematic approach to classifying terrain based on its potential avalanche exposure, relying exclusively on static terrain and vegetation characteristics. While early ATES maps were manually produced by experts, recent advancements, such as machine learning-based approaches, have enabled scalable and accurate automated ATES (AutoATES) mapping over larger areas. A critical component of AutoATES classification involves modelling the protective effect of forests, which requires information on forest structure. Recent applications have incorporated remote sensing-derived forest structural parameters, such as percent canopy cover (PCC), to approximate forest protective effects in AutoATES model chains. In this study, we applied a recently developed machine learning framework for AutoATES classification to generate ATES maps for a study area in the Pirin Mountains, focusing on the effect of different PCC datasets on final and intermediate results of the model chain. Specifically, we tested a few forest cover scenarios: (1) the Copernicus Tree Cover Density product as a baseline, (2) a treeless simulation with PCC set to 0%, and (3) two alternative PCC estimates derived from RapidEye satellite imagery and information from digital elevation models. Our findings indicate that forests significantly reduce avalanche terrain exposure in the study area and that variations in PCC estimation can substantially alter the extent and distribution of different ATES classes. Notably, the second investigated RapidEye-based PCC estimation approach

provided improved identification of forest gaps and dwarf mountain pine (*Pinus mugo* Turra) compared to the first, resulting in more accurate ATES maps in those respective areas.

Key words: Copernicus Tree Cover Density, machine learning, protective effect of forests, RapidEye, snow avalanches.

Introduction

Snow avalanches are estimated to cause approximately 250 fatalities worldwide each year (Acharya et al. 2023). Bulgaria has experienced a sharp rise in back-country recreation, accompanied by regular avalanche accidents (Panayotov et al. 2021).

Improving public and institutional awareness of avalanche-prone terrain is critical for mitigating avalanche risk and enhancing safety. One effective tool for this is the Avalanche Terrain Exposure Scale (ATES), which classifies terrain based on its potential exposure to avalanche hazards. ATES maps rely solely on static terrain and vegetation characteristics, and do not incorporate dynamic meteorological or snowpack conditions. Originally developed in Canada in 2006 (Statham et al. 2006), its first version categorised terrain into the following three classes – simple, challenging, and complex. A fourth class, extreme, was later added in the second version to account for high-consequence avalanche terrain, such as steep gullies, cliffs or vertical drop-offs (Statham and Campbell 2025).

Initial ATES maps were produced manually by avalanche professionals and relied on expert-based assessments of the local avalanche terrain (Campbell and Gould 2013, Gavalda et al. 2013). While effective, this process is labour-intensive and difficult to scale across large or remote areas. To address this limitation, au-

tomated approaches such as AutoATES versions 1.0 and 2.0 (Larsen et al. 2020, Toft et al. 2024) and their derivatives (Avis et al. 2023, Huber et al. 2023, Sykes et al. 2023, Markov and Panayotov 2024, Markov et al. 2024) have been developed. The most recent advancements include the use of machine learning approaches that combine multiple geospatial data layers to create ATES maps (Markov et al. 2025).

Regardless of the method used to generate ATES maps, accounting for the protective effect of forests is essential and results in improved accuracy (Schumacher et al. 2022). Forests are widely recognised as providing significant biological protection against avalanches. When assessing this protective role, it is important to differentiate between avalanche release areas and runout zones. In release areas, forests can mitigate the likelihood of avalanche release, although the degree of protection depends on multiple factors, including stem density, crown cover, forest gaps distribution, terrain ruggedness relative to snow depth, and other structural characteristics (Bebi et al. 2009). The influence of forests on avalanche runouts has also been extensively studied. Research indicates that the runout distances of small to medium avalanches are particularly affected by forest structure. In contrast, medium to large avalanches originating above treeline are generally less influenced by forest structure; however, their speed and energy often decreases upon entering forested terrain (Teich

et al. 2012). Beyond avalanches, forests also serve as effective natural barriers against other hazards such as rockfalls and landslides. Given their multifunctional role in hazard mitigation, understanding forest characteristics and quantifying their protective capacity are essential for comprehensive natural hazard risk assessments (Perzl et al. 2021). Usually, most existing approaches utilise remotely sensed forest structure information to assess the protective effects of forest vegetation. Among other metrics Percent Canopy Cover (PCC) can be estimated based on data from different remote sensing platforms and has been used to characterise the forest protective effect of forests against avalanches.

In this study, we adopted the machine learning framework developed by Markov et al. (2025) to create ATES maps for a part of the Pirin Mountains, Bulgaria. The main objective was to investigate how forest PCC layers derived from open source and easily accessible data sources affect the identification of potential avalanche release areas (PRAs), modelled avalanche runouts and intensities, and the resulting ATES classifications. In the first step, Copernicus Tree Cover Density data (Copernicus Land Monitoring Service 2018), as originally used by Markov et al.

(2025), was adopted. Next, we simulated a treeless landscape by setting PCC to 0 % across the region to observe the complete loss of protective effect. Finally, we tested two alternative methods for estimating forest PCC, inspired by the recent work of Panayotov et al. (2024), using RapidEye satellite imagery (European Space Agency 2025) and a combination of digital surface (DSM) and digital terrain models (DTM).

Through this investigation, we aimed to address two main research questions:

1. Do forests in the study region significantly mitigate avalanche hazard through their protective effect?

2. How do different estimates of PCC created from easily accessible data sources influence resulting ATES map quality compared to maps produced with the Copernicus Tree Cover Density product as a proxy for PCC?

Materials

Our study focused on an area around Bansko Ski Resort and the neighbouring Bunderitsa and Demyanitsa Valleys, located just to the south of the town of Bansko (Fig. 1). These valleys are representative of the entire northern part of the

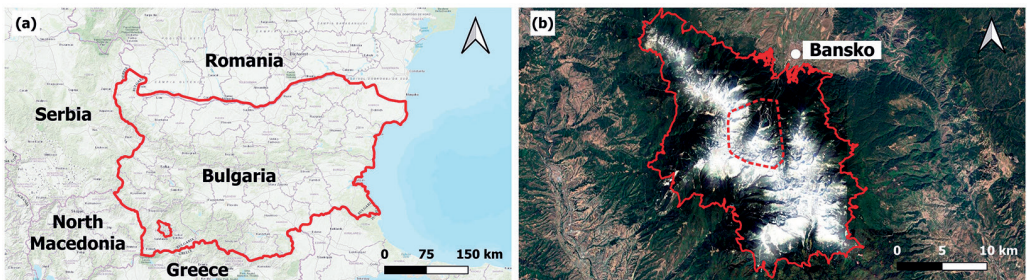


Fig. 1. Location of the study area: (a) location of Pirin National Park in southwestern Bulgaria; (b) study area shown as a dashed red line within Pirin National Park (solid red line).

Pirin Mountain range and contain sharp peaks with steep slopes and numerous avalanche couloirs and tracks below them. Some of these avalanche paths run through forests and avalanches have occurred there in the past (Panayotov et al. 2023, Panayotov and Tsvetanov 2024). The lower sections of the valley slopes (up to approx. 2200 m a.s.l.) are predominantly covered by Macedonian pine (*Pinus peuce* Griseb.)-dominated forests, while in extensive areas of the middle and upper parts of the valleys, up to approximately 2500 m a.s.l., dwarf mountain pine (*Pinus mugo* Turra) dominates the landscape. The mountain tops are free of high vegetation and are covered mostly by bare rocks, low juniper bushes (*Juniperus communis* L. var. *nana* Rehder) and grasses, located on steep to very steep slopes, sometimes with sections of vertical cliffs.

In this study, our analysis primarily concentrated on ATES classifications within forested regions and areas dominated by dwarf mountain pine, which constitute a large portion of the study area and exhibit the most pronounced differences across the various methods used to estimate the PCC.

The input data requirements for this study closely align with those used in previous studies (Larsen et al. 2020, Toft et al. 2024). The two primary datasets needed are a DTM and a forest structure layer. In many studies PCC has been used to approximate forest structure in terms of forest density. The DTM used for most of the study area had a resolution of 5 m and was provided by Pirin National Park (2022). Additionally, a higher-resolution 2 m DTM from the private geospatial provider GEO Polymorphic Cloud (2025) was used for a limited portion of the study area. To ensure consistency with previous AutoATES stud-

ies, both DTMs were combined and resampled to a resolution of 10 m. This resolution has been found to provide a good balance between processing time, amount of noise in the data (arising from terrain irregularities such as small isolated rocks or microtopographic features), and the level of geographical detail required to capture major terrain features.

For the representation of forest structure, we investigated different data sources describing PCC in our study area. The first approach utilised the readily available 10 m resolution Copernicus Tree Cover Density product (Copernicus Land Monitoring Service 2018), hereafter referred to as Copernicus PCC for consistency with previous AutoATES related studies. In addition, we also investigated two approaches for custom PCC estimation, which were inspired by the work of Sykes et al. (2022) and Panayotov et al. (2024). These approaches made use of RapidEye satellite imagery (European Space Agency 2025), with a resolution of 5 m, which provides five spectral bands: blue, green, red, red edge, and near-infrared. A DSM obtained from the Bulgarian Ministry of Agriculture and Food, with a resolution of 4 m, was also employed for the second approach.

All datasets were reprojected to the WGS 1984 UTM Zone 35N coordinate reference system. For the two custom PCC estimation approaches, the pixel size was resampled to match the highest native resolution among the input data layers used in each case – 5 m for the first approach and 4 m for the second. The resulting PCC layers were subsequently resampled to a 10 m resolution to ensure consistency with the DTM before further processing. All resampling was performed using bilinear interpolation in ArcGIS Pro. Spatial alignment between layers was ensured using the Extract by Mask tool.

Methods

General methodology

The methodology used to generate the ATES maps followed the machine learning approach described by Markov et al. (2025), closely resembling the general structure adopted in previous AutoATES model chains (Larsen et al. 2020, Huber et al. 2023, Panayotov et al. 2024, Toft et al. 2024). The standard modelling workflow consists of three main steps: (1) identification of PRAs, (2) delineation of potential avalanche runouts from those PRAs, and (3) a final avalanche terrain classification step, often referred to as the AutoATES classifier (Toft et al. 2024). All basic steps and parameter settings were replicated exactly from the study by Markov et al. (2025). The remainder of this section provides a summary of this approach.

The methodology for identifying PRAs originates from the approach proposed by Veitinger et al. (2016) and used the input layers slope, wind shelter (both calculated from the DTM), and PCC, converting them to the interval of 0 to 1 using fuzzy logic. These input layers are then combined and weighted by a fuzzy logic approach into a single continuous PRA value, representing the likelihood of avalanche release from 0 to 1. The resulting continuous PRA layer was subsequently converted into binary format using a threshold value of 0.3, consistent with previous studies (Huber et al. 2023, Panayotov et al. 2024, Toft et al. 2024, Markov et al. 2025). In this binary layer, a value of 1 indicates that avalanche release is possible at a given location ($PRA > 0.3$), while a value of 0 indicates that it is not.

To model potential avalanche runouts, the simulation tool com4FlowPy with its forest friction module was used (D'Am-

boise et al. 2022, Huber et al. 2024). The binary PRA layer from the previous step served as the potential release area input, along with the same DTM used in that calculation. The forest structure index (FSI) input into the software was derived by linearly scaling the PCC, which originally ranges from 0 % to 100 %, to the interval 0 to 1. The FSI represents the degree to which a forest with a given structure is capable of decelerating avalanches, where a value of 1 indicates an optimal forest structure for protection and 0 indicates the absence of forest. The com4FlowPy outputs used in this study were the local travel angle (γ), cell counts, $z_{\max}^{\delta}$, and the sum of routed flux ($\sum_{\text{routFlux}}$). Local travel angle provides a measure of the proximity of a location to the nearest PRA. Cell counts represents how many potential PRA cells may affect a given location. The $z_{\max}^{\delta}$ layer indicates the maximum avalanche intensity at a given cell (which can be converted to velocity), while $\sum_{\text{routFlux}}$ represents the sum of all modelled flux values. $\sum_{\text{routFlux}}$ can be interpreted more intuitively as potential flow thickness at a certain point along an avalanche path. Consequently, higher modelled flux values are observed in gullies or locations where multiple avalanche paths may converge.

The final ATES classification step combines outputs from the previous steps of the model chain and maps them to the four ATES classes. This final classification step was performed using the best performing Random Forest model (model RF 2) from the study by Markov et al. (2025).

Study-specific methodology

This study specifically focused on evaluating the influence of different PCC representations on the PRA outputs and the final ATES classification. It is important

to note that each PCC implementation used in this study was applied consistently across all components of the model chain – including the PRA calculation, avalanche runout and intensity modelling with com4FlowPy, and the final classification step. This consistency allowed us to investigate the influence of each PCC layer on all intermediate data layers as well as on the final ATES map.

The baseline scenario replicated the entire setup from Markov et al. (2025), using Copernicus PCC and the best-performing Random Forest model from that study (model RF2). In the first analysed scenario we replaced the PCC layer with a synthetic raster containing zero values across the entire domain. This analysis was conducted to address Research Question 1 by modelling the avalanche terrain exposure in our study area in the absence of forest cover. We refer to this scenario as the NoForest scenario.

In the next step we investigated two alternative methods to derive custom PCC layers and assessed how they impact both PRA detection and final ATES outputs. The first approach followed a method outlined by Sykes et al. (2022), using supervised machine learning classification and RapidEye imagery. We manually created training polygons for forest and non-forest classes by visual inspection of high-resolution orthophoto imagery and converted them to a raster layer (with 0 representing forest and 1 representing non-forest), splitting them at random (50/50) into a train/test set partition. We used the five spectral bands of the RapidEye imagery along with three derived indices – the Normalized Difference Vegetation Index (NDVI), Normalized Difference Water Index (NDWI), and Normalized Difference Red Edge Index (NDRE) as input features for the classification, as

described in Sykes et al. (2022). NDVI helps distinguish healthy vegetation from bare soil or rock, while NDWI detects water bodies. NDRE highlights chlorophyll content for assessing subtle vegetation health. All three indices provide valuable information for forest vs non-forest classification and supplying them to a machine learning model leverages the unique strengths of each. A Random Forest classifier was trained to distinguish between the forest and non-forest classes using a hyperparameter grid identical to that of the Random Forest models in Markov et al. (2025), with 500 random combinations evaluated via 3-fold cross-validation. The resulting model was then applied on the held-out test set and on the remainder of the study area to classify them into forest and non-forest classes. This approach is hereby referred to as the RapidEye approach.

The second approach from RapidEye imagery shares the same methodology as the first, but was extended by incorporating an additional feature into the classification – the difference between a DSM and a DTM. The applied methodology is also described in Panayotov et al. (2024). The DSM-minus-DTM feature was used as an additional input to better distinguish forested areas from regions dominated by dwarf mountain pine – a known area of confusion. Dwarf mountain pines and higher conifer tree species are spectrally similar, leading to frequent misclassifications using the RapidEye approach. However, dwarf mountain pine, while dense, is much shorter than typical trees and frequently becomes entirely buried under snow in winter, thus losing its protective effect against avalanches. Correctly identifying and separating it from taller forest vegetation is therefore of high importance. Large differences between the DSM and

the DTM suggest forested areas, while dwarf mountain pine is significantly shorter than trees, resulting in a small difference there. This approach is hereby referred to as the RapidEye Enhanced approach.

We made a brief comparison of the binary forest vs non-forest classification performance of both custom approaches by using the held-out test set, which was not seen at all during training procedures. We used the commonly applied statistical measures precision, recall, and F1 score for each class, as well as the corresponding precision-recall curves. Precision quantifies the proportion of correctly classified instances among all instances predicted as belonging to a given class. Recall, on the other hand, measures the proportion of actual instances of a class that were correctly identified by the model. The F1 score is a measure of a model's accuracy that combines precision and recall and is especially useful in the case of class imbalances. The precision-recall curve illustrates the trade-off between precision and recall across different decision thresholds, with better-performing models producing curves that stay close to the top-right corner.

Next, it was necessary to convert the resulting binary forest vs non-forest classification from both custom approaches into a PCC layer representing forest density, ranging from 0 % to 100 %, just like the Copernicus PCC. This was done using a 3×3 pixel sliding window method, as described by Sykes et al. (2022). The sliding window method counts the number of pixels classified as forest within each sliding window and assigns the resulting proportion (expressed as a percentage) to the central pixel. After the conversion from a binary layer to PCC, the FSI again was derived by linearly scaling this PCC to the interval 0 to 1, as was done with the base-

line Copernicus PCC approach.

After obtaining the PCC from both RapidEye approaches and using it to calculate the PRAs and avalanche runouts using com4FlowPy, the final step was to integrate all features into the final ATES map. For this stage, we utilised the best-performing Random Forest model identified by Markov et al. (2025), hereafter referred to as RF2. This model incorporates a range of input features – slope, PCC, a continuous raster representing the potential release areas (PRA), and the previously described outputs from the com4FlowPy model. We retrained the model using feature values generated from our two alternative RapidEye PCC datasets specific to this study. Each retraining was conducted using the same training set and an identical hyperparameter grid search space, with 10,000 randomly sampled combinations, consistent with the original methodology described in Markov et al. (2025). For the NoForest scenario, we did not retrain the model – instead, we applied the pre-trained RF2 model from Markov et al. (2025) directly, as this scenario was intended solely to test a hypothetical situation in which no forest cover is present.

We formally compared the results for the final ATES classification stemming from Copernicus PCC (Markov et al. 2025), the NoForest scenario, and from our two alternative RapidEye PCC approaches (RapidEye, RapidEye Enhanced) by calculating the precision, recall, and F1 score for each ATES class and analysing corresponding precision-recall curves in the held-out test set. This set was identical to the one used in the study by Markov et al. (2025) and was kept the same for each approach in our study to ensure consistency and comparability of the results.

In addition, we conducted a visual in-

spection of the PRA and final ATES results for selected regions of interest to evaluate how the classifiers based on the different PCC layers perform beyond the test set.

Results

Forest classification and PRAs

Figure 2 illustrates the test set performance for the forest vs non-forest classification step in both custom PCC approaches. The DSM-minus-DTM feature in the RapidEye Enhanced approach emerged as an influential variable, leading to a notable improvement in classification performance on the test set. This enhancement is reflected in the precision-recall curves for each class (Fig. 2a), as well as in the overall precision, recall, and F1 scores (Fig. 2b) – all of which show significant improvements with the enhanced method. In Figure 2a it can be seen how the

precision-recall curves for the RapidEye Enhanced approach are very close to the ideal curve, indicating that the classifier is highly confident in its classification on the test set. These improvements in forest classification are subsequently reflected in the more accurate results observed in the downstream ATES classification (Fig. 4c).

Figure 3 presents the PRAs as a binary layer, focusing on a region encompassing the western slope of Todorka Peak and the Echmishte area, located centrally within Bansko Ski Resort. This region covers a heterogeneous landscape consisting of forest openings and gaps, ski trails, couloirs, and extensive cover of dwarf mountain pine. Figure 3a displays the PRA results based on the baseline approach using Copernicus PCC. Figures 3b and 3c show the results using the RapidEye approach and the RapidEye Enhanced approach, respectively. Figure 3d illustrates the hypothetical scenario in

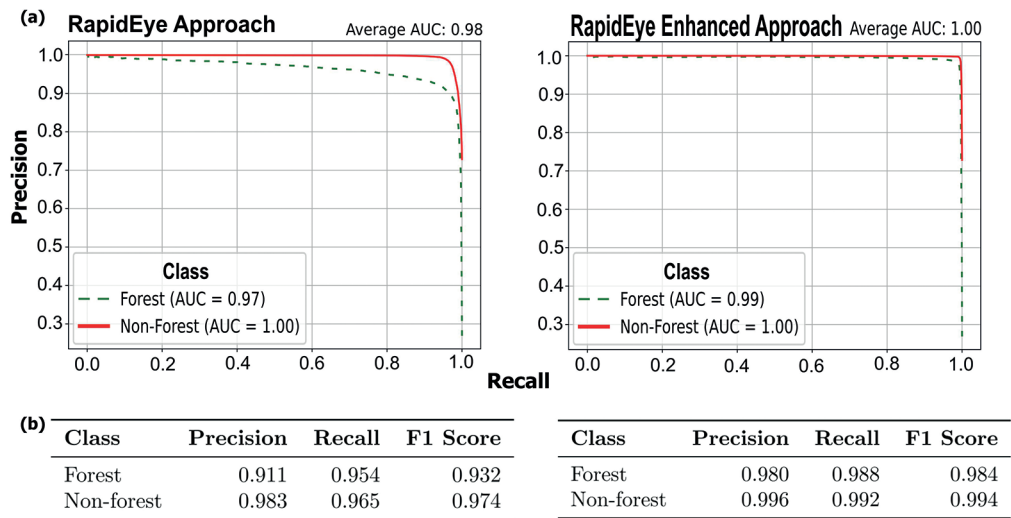


Fig. 2. Test set performance for forest/non-forest classification using the RapidEye and RapidEye Enhanced approaches: (a) precision–recall curves; (b) precision, recall, and F1 scores for both classes.

Note: AUC denotes the area under the curve.

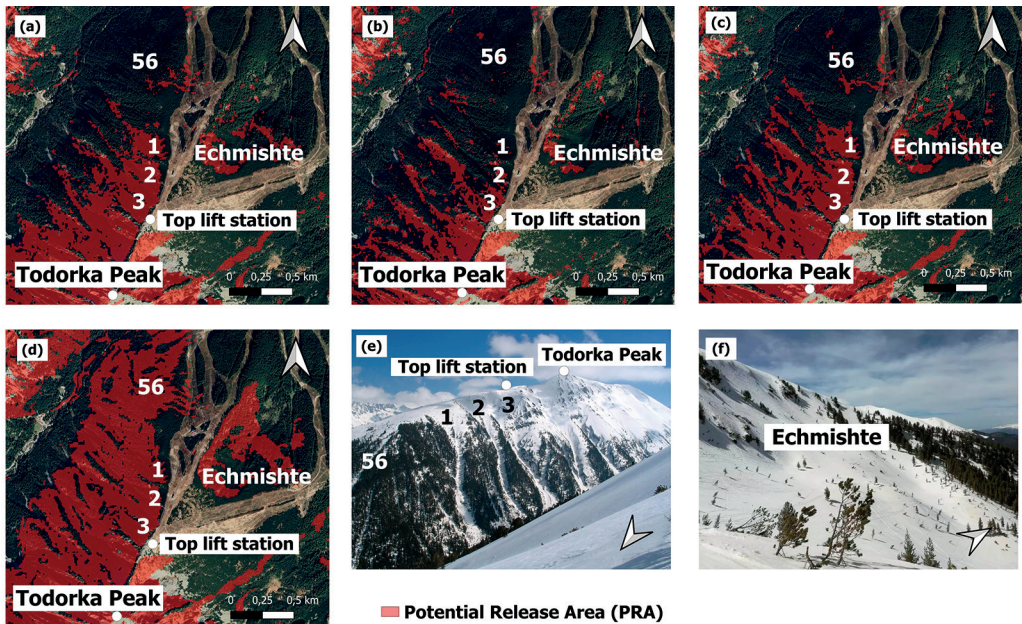


Fig. 3. Potential release areas (PRAs) calculated under different forest PCC scenarios: (a) Copernicus PCC; (b) RapidEye; (c) RapidEye Enhanced; (d) NoForest (0% PCC).

Note: Panel (e) shows a wintertime photo of the western face of Todorka Peak, panel (f) – a wintertime photo of the Echmishte region within Bansko Ski Resort. Numbers indicate corresponding locations in panels (a)–(e).

which forest cover is entirely absent (the NoForest scenario). For visual reference, figures 3e and 3f include real-world photographs of the region.

The figure highlights that, in the absence of forest cover (NoForest scenario) a significant portion of the area would be classified as PRAs (Fig. 3d, red colours). Additionally, the standard RapidEye approach (Fig. 3b) fails to correctly identify areas dominated by dwarf mountain pine – such as Echmishte and the top sections of couloirs 1, 2, and 3 on the western slope of Todorka Peak – misclassifying them as forest. Consequently, large parts of these areas are excluded from PRAs, which does not reflect observed local conditions. In contrast, the RapidEye Enhanced approach (Fig. 3c) is more successful in

distinguishing dwarf mountain pines from areas covered by taller forest vegetation. The resulting PRAs closely correspond to those derived with the baseline Copernicus PCC result (Fig. 3a).

Final ATES classification

Figure 4 displays the final ATES maps for the same region, with panel labels corresponding to those in Figure 3. In the NoForest scenario (Fig. 4d), a substantial portion of the terrain is classified as complex, in contrast to the simple or challenging classifications produced by the other approaches. The standard RapidEye approach (Fig. 4b), due to its misclassification of extensive areas of dwarf mountain pine as forest, erroneously la-

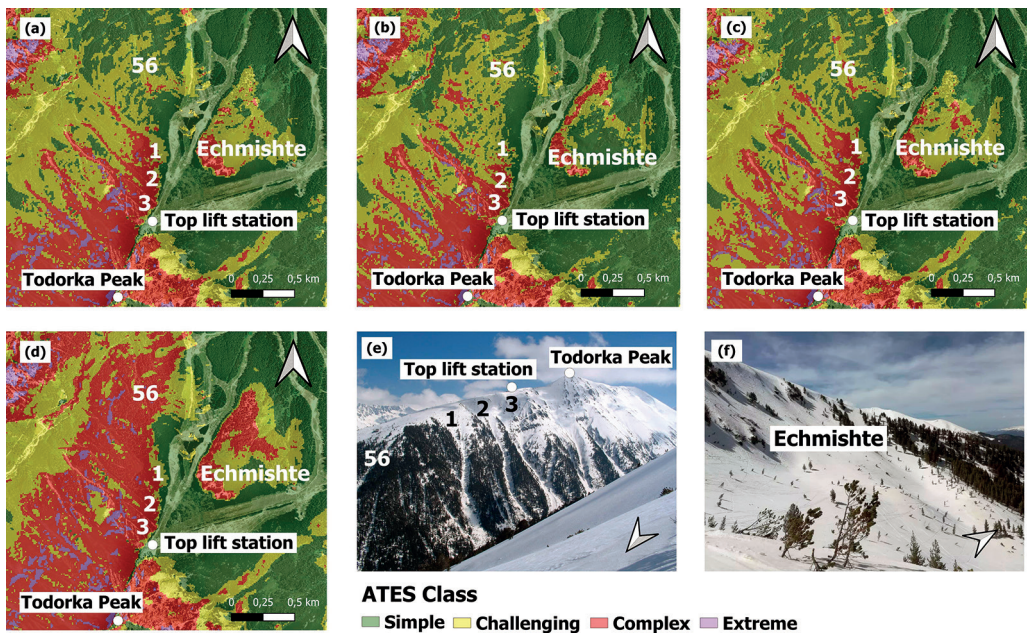


Fig. 4. ATES maps calculated under different forest PCC scenarios: (a) Copernicus PCC; (b) RapidEye; (c) RapidEye Enhanced; (d) NoForest (0% PCC).

Note: Panel (e) shows a wintertime photo of the western face of Todorka Peak, panel (f) – a wintertime photo of the Echmishte region within Bansko Ski Resort. Numbers indicate corresponding locations in panels (a)–(e).

bels larger sections of the Echmishte region as simple terrain. It also assigns less severe terrain classifications to the upper parts of couloirs 1 and 2 on the western slope of Todorka Peak - areas that are steep, frequently serve as avalanche start zones, and should be classified as complex terrain, as accomplished by the other approaches. In contrast, the ATES maps generated using Copernicus PCC (Fig. 4a) and the RapidEye Enhanced approach (Fig. 4c) are both accurate and very similar, with the latter offering slightly improved delineation of narrower forested corridors in some areas.

Figure 5 displays the feature importance derived from training the Random Forest models for final ATES classification using the Copernicus PCC (as in Markov et al. 2025), the RapidEye approach PCC

and the RapidEye Enhanced approach PCC. The PCC feature ranks lowest in importance in all approaches, indicating that it was used in a very small proportion of splits in the trained models. In contrast, the continuous PRA feature and all com4FlowPy outputs, which are directly influenced by the PCC, exhibit significantly higher importance in all three models. Slope and the sum of routed flux are identified as the two most important features in all models. The continuous PRA layer, the local travel angle (γ), and cell counts are identified as the next most important features (albeit in varying order across the three models), being used in approximately 5–15 % of splits for both models.

Figure 6a shows the precision-recall curves on the test set for final ATES classification using the Copernicus PCC and

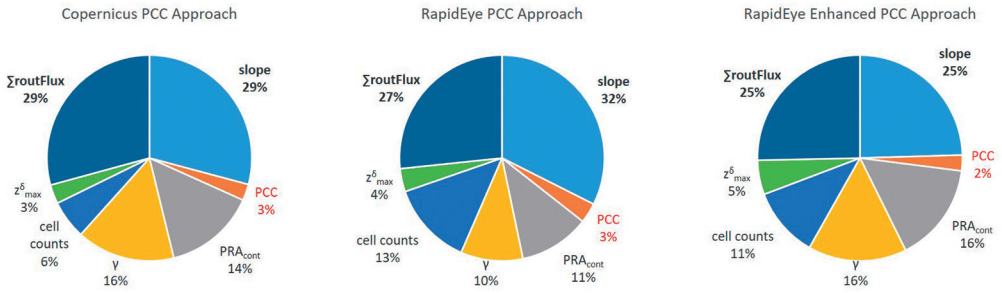


Fig. 5. Feature importance derived from training the Random Forest models for final ATES classification, using input features obtained from the Copernicus and the two RapidEye imagery-based approaches for PCC calculation.

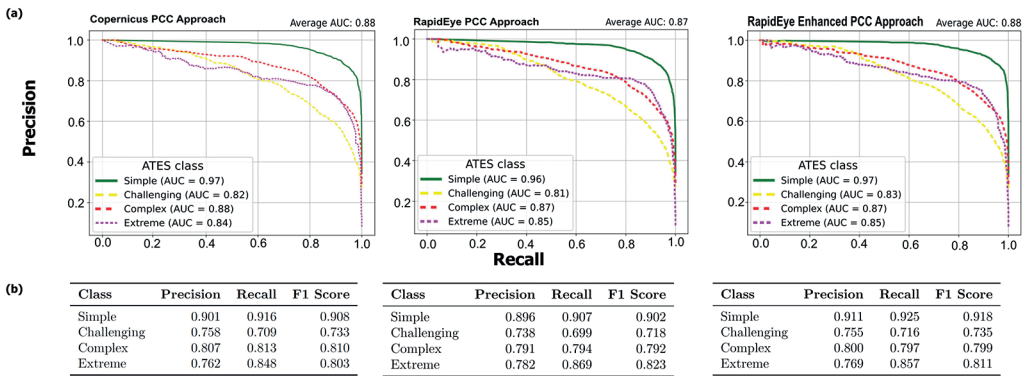


Fig. 6. Test set performance for the final ATES classification with Copernicus PCC and PCC obtained using the RapidEye approach and the RapidEye Enhanced approach: (a) precision–recall curves; (b) actual values of precision, recall, and F1 score for all ATES classes.

the two custom PCC approaches. We observe that performance is slightly lower for the RapidEye approach for PCC across most classes when compared to the RapidEye Enhanced PCC approach. Performance of the RapidEye Enhanced approach and the Copernicus baseline approach (Markov et al. 2025) is similar on the test set. Figure 6b presents the per-class precision, recall, and F1 scores for the models using Copernicus PCC and using the custom RapidEye approaches PCC. The RapidEye Enhanced approach yields a modest improvement in test set

performance compared to the RapidEye approach across all ATES classes except for the extreme class, where a slight decline is observed. This misclassification becomes evident from the confusion matrices for the two custom PCC models (tables 1 and 2). For each of the four ATES classes, the confusion matrices display how many samples were correctly classified and how many were misclassified into each of the other classes, allowing assessment of classification accuracy and error patterns. The numbers along the diagonal reveal correct classifications

Table 1. Confusion matrix for the final ATES classification using PCC obtained with the RapidEye approach.

Actual ATES class, m ²	Predicted ATES class			
	Simple	Challenging	Complex	Extreme
Simple	408,300 (90.67 %)	37,700 (8.37 %)	4200 (0.93 %)	100 (0.02 %)
Challenging	43,200 (12.09 %)	249,700 (69.89 %)	62,200 (17.41 %)	2200 (0.62 %)
Complex	3700 (0.96 %)	50,900 (13.25 %)	304,900 (79.38 %)	24,600 (6.40 %)
Extreme	300 (0.27 %)	0 (0.00 %)	14,200 (12.82 %)	96,300 (86.91 %)

Note: Grey diagonal cells represent correctly classified areas (values given in m²; dividing by 100 yields the number of pixels), while off-diagonal entries indicate misclassifications.

Table 2. Confusion matrix for the final ATES classification using PCC obtained with the RapidEye Enhanced approach.

Actual ATES class, m ²	Predicted ATES Class			
	Simple	Challenging	Complex	Extreme
Simple	416,500 (92.49 %)	32,800 (7.28 %)	1000 (0.22 %)	0 (0.00 %)
Challenging	38,500 (10.78 %)	255,900 (71.62 %)	60,600 (16.96 %)	2300 (0.64 %)
Complex	1900 (0.49 %)	49,700 (12.94 %)	306,300 (79.74 %)	26,200 (6.82 %)
Extreme	300 (0.27 %)	700 (0.63 %)	14,800 (13.36 %)	95,000 (85.74 %)

Note: Grey diagonal cells represent correctly classified areas (values given in m²; dividing by 100 yields the number of pixels), while off-diagonal entries indicate misclassifications.

(where the actual and predicted ATES classes match). The matrices reveal that for the RapidEye approach, 12.82 % of the actual extreme terrain pixels were misclassified as complex, while for the RapidEye Enhanced approach, this percentage increased slightly to 13.36 %. However, when visually evaluating model generalisation over larger spatial extents (Fig. 4), the RapidEye Enhanced approach demonstrates substantially improved accuracy compared to the RapidEye approach, closely aligning with the baseline Copernicus PCC model and even surpassing it in certain aspects – particularly in the detection and delineation of narrower forest openings. The performance metrics (precision, recall, and F1 score) of the Copernicus PCC model and the RapidEye Enhanced PCC model are also very similar, with slight improvements for

the simple and extreme terrain classes in the latter.

Discussion

The results of this study provide clear evidence in support of the research questions outlined at the beginning of the paper. Figures 3a, 3d and 4a, 4d illustrate the substantial protective effect of the forest cover in the study area and its critical role in mitigating avalanche hazard. As shown in Figure 3d, the extent of PRAs would be considerably larger in the absence of the forest cover (NoForest scenario). This finding aligns with established knowledge, as sufficiently dense forest cover is known to exert a stabilising influence on the snowpack, thereby inhibiting the release of avalanches in forests (Bebi et al. 2009,

Bebi et al. 2021). The PRAs, together with the FSI – defined as the scaled PCC – serve as inputs to com4FlowPy, directly influencing modelled avalanche runouts and intensities.

In Figure 4, this influence on ATES classes becomes evident: in the NoForest scenario (Fig. 4d), a large portion of the terrain is classified as complex, whereas with existing forest cover (Fig. 4a, 4b, 4c), much of the same area is more accurately identified as simple or challenging terrain (challenging being the areas that are very steep). These findings align with the earlier PRA results and are consistent with the known role of dense forests in reducing avalanche hazard (Bebi et al. 2009, Teich et al. 2012, Perzl et al. 2021). Generally, densely forested terrain is considered low-risk and classified as simple terrain, unless it includes steep slopes with forest gaps and openings, in which case it is appropriately labelled as challenging, which is consistent with Figure 4a, 4b, 4c.

The results of this study also allow us to examine the impact of different PCC estimation methods on modelled PRAs and the final ATES maps. As shown in Figure 2, the RapidEye Enhanced approach significantly improves the initial forest vs non-forest classification performance compared to the base RapidEye approach. This aligns with the findings of Panayotov et al. (2024), where incorporating the DSM-DTM difference led to more accurate delineation of forested and non-forested terrain. The DSM-minus-DTM feature emerged as the most important variable during the model training, which is expected, as areas without tall forest vegetation – such as dwarf mountain pine zones, open alpine terrain, and forest openings – tend to have low DSM-minus-DTM values, whereas tall trees produce high differences, making this fea-

ture particularly discriminative.

Figures 3b, 3c and 4b, 4c illustrate how errors in the initial forest classification propagate downstream to PRA detection and ATES terrain classification. In particular, the omission of the DSM-minus-DTM feature in the original RapidEye approach results in widespread misclassification of dwarf mountain pine as dense forest. Consequently, extensive areas in the upper sections of couloirs 1, 2, and 3 on the west face of Todorka, as well as in the Echmishte region are excluded from PRA maps and assigned a simple or challenging ATES classification. While such classification would be appropriate if these areas were indeed densely forested, in reality they are highly avalanche-prone and should be classified as complex. This visual comparison highlights that the RapidEye PCC approach produces lower-quality PRA and ATES maps compared to the Copernicus PCC baseline (figs 3a and 4a), which correctly identifies these regions as complex.

The RapidEye Enhanced PCC approach (figs 3c and 4c) provides a solution to the above-mentioned problem, correctly classifying dwarf mountain pine regions and producing results that closely match those of the Copernicus PCC baseline (figs 3a and 4a), in some cases even surpassing them. Although the overall maps are similar, the RapidEye Enhanced method better captures narrow forest couloirs and small forest gaps – such as the couloir 1 and gap 56 – thanks to its original use of higher-resolution input (5 m DTM, 4 m DSM) and the inclusion of the DSM-minus-DTM feature when calculating the PCC. These advantages help to better delineate small open-terrain features within forests and track them downslope, something that Copernicus PCC (with its coarser 10 m resolution) struggles with. It

is important to note that the subsequent resampling of the PCC layer in the RapidEye Enhanced approach to 10 m resolution before the further processing likely resulted in a minor loss of detail. Despite this, figures 3c and 4c show that the resulting PRA and ATES maps still reflect the advantages gained from initially using the finer-resolution data.

In open, alpine areas above the treeline and above the dwarf mountain pine regions, ATES classification is very similar across all investigated methods, as all PCC approaches produce the same value of 0% PCC in most locations in these areas.

Feature importance analysis for the final ATES classification Random Forest models (Fig. 5) further confirms findings from Markov et al. (2025) – slope and the sum of routed flux are consistently the most important features, while the PCC layer ranks as the least important. This strengthens the conclusion from that study that, since PCC is already used in defining PRAs and influences all com4FlowPy outputs, which are subsequently used in the final ATES classification step, it may be redundant to include it as a separate feature at that stage. In this study, regardless of the source, that PCC layer consistently showed very low importance, offering further support for its exclusion from this step.

Furthermore, formal model comparisons for the final ATES classification (Fig. 6) show that the RapidEye Enhanced approach for PCC on average outperforms the original RapidEye approach on the test set, though the improvement is modest (approximately 1–2 % on average). However, there is a slight decrease in performance in the RapidEye Enhanced approach for the extreme ATES terrain class. The decrease may stem from the

fact that the DSM and DTM may not be of as high quality over extreme terrain (very steep slopes and cliffs) and may thus exhibit large differences in those areas. This may have misled the model, and a small portion of this extreme terrain could have been incorrectly labelled as forested during the PCC stage of the processing chain, thereby being assigned a lower final ATES class. As shown in the confusion matrices in tables 1 and 2, the proportion of underclassified extreme terrain pixels was 13.09 % (with most being misclassified as complex) in the RapidEye approach, while in the RapidEye Enhanced approach, the proportion of underclassifications for the extreme class was 14.26 %, again with most errors falling in the complex class. This increase in misclassifications is relatively minor, considering the improvements in classification across all three other ATES classes.

The comparison between the RapidEye Enhanced approach and the Copernicus PCC-based method used in Markov et al. (2025) demonstrates very similar performance across all statistical metrics. This result is encouraging, as it provides mutual validation of both methodologies and supports their reliability. In contrast, a discrepancy between the two would have raised concerns regarding the accuracy of one or both approaches. Moreover, the RapidEye Enhanced approach is comparatively simpler, utilising eight input features derived from one input image (plus the DSM-minus-DTM feature from digital elevation models), whereas the Copernicus PCC method relies on spectral features extracted from multi-temporal imagery and employs a complex model chain, with several post-processing steps (Copernicus Land Monitoring Service 2018). Despite this difference in methodological complexity, our RapidEye Enhanced approach achieves

comparable results. Both approaches yield an average area under the precision-recall curve (AUC) of 0.88 for the final ATES classification, with per-class AUC values either identical or differing by no more than 1 %. These findings indicate that the RapidEye Enhanced method offers a reliable alternative to the Copernicus PCC dataset as input for ATES classification and can be applied in regions where Copernicus PCC is not openly available, such as outside Europe. Furthermore, as previously noted, our visual inspection suggests that the RapidEye Enhanced PCC-based ATES classification may outperform the Copernicus PCC-derived map in certain areas (such as forest gaps), although formal statistical validation is required to confirm this observation.

Conclusion and future work

This study demonstrates that forests in our study area in the Pirin Mountains (southwestern Bulgaria) have a notable protective effect against snow avalanches. The extent of the potential release areas would be significantly larger in the absence of forest cover, and a greater portion of the terrain would be assigned the complex ATES class.

We also find that alternative methods for estimating PCC using RapidEye satellite imagery, DEMs, and Random Forest models can provide a viable alternative to the Copernicus PCC as input for ATES classification, particularly in regions where Copernicus PCC is unavailable, such as areas outside Europe. Although the initial RapidEye approach often fails to distinguish dwarf mountain pine from taller forest, thereby reducing ATES classification performance, our RapidEye Enhanced method effectively utilises available DSMs and DTMs to resolve this issue,

producing final ATES results that closely match those derived from the Copernicus PCC baseline. In some cases, the RapidEye Enhanced approach even appeared to outperform the Copernicus PCC approach in identifying and tracking narrow forest openings.

While this study provides valuable insights, several opportunities for future work remain. The test set used to evaluate the final ATES classification, sourced from Markov et al. (2025), is representative of the overall terrain but was not specifically designed to target forested areas or regions dominated by dwarf mountain pine. Creating a test set that more directly focuses on these vegetation types could improve the ability to detect subtle differences between PCC estimation methods and better align visual observations with quantitative metrics.

Additionally, this study applied existing methods for PCC estimation rather than developing new ones. Future research could explore innovative approaches for generating forest PCC layers, potentially allowing more precise identification of forest openings, gaps, and couloirs – key elements for improving PRA detection and refining ATES terrain classification in complex environments.

Finally, rather than resampling the PCC outputs derived from higher-resolution layers to 10 m, as was done in both the RapidEye approach and the RapidEye Enhanced approaches, future work could consider retaining the original resolution throughout the processing chain to preserve the full level of spatial detail.

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Phenological expression of sweet cherry cultivars grafted onto Maxma 14 rootstock in Southern Bulgaria

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Thematic field: Agriculture in mountainous and semi-mountainous regions

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Abstract

Phenological observations are among the most important for identifying the responses of fruit trees to climatic conditions of their habitat. Temperature changes have most significant influence on the phenology of fruit trees, particularly on flowering. This study aimed to monitor the phenological development of six sweet cherry cultivars grafted onto Maxma 14 rootstock. Phenological phases, including bud swelling (BBCH 51), full flowering (BBCH 65), and fruit harvest maturity (BBCH 87), were monitored to determine the duration of flowering, length of growing season, and the period from full flowering to fruit harvest maturity. Changes at the beginning of the growing period, occurring 2 to 8 days earlier in the second reporting year, were observed for most of studied cultivars. Deviations were also noted in the phenological phase BBCH 65, which occurred 4–8 days earlier and was 2–5 days shorter in the second year. This study demonstrated that temperature changes significantly impacted both duration and timing of the flowering and growing seasons in sweet cherries.

Key words: *Prunus avium*, phenology, temperature, monitoring, vegetation period.

Introduction

Sweet cherry (*Prunus avium* L.) is a widely cultivated species, present in approximately 70 countries and regions around the globe (Habib et al. 2017), including Europe, North and South America (Iez-

zoni 2008), Asia, Africa, and Oceania (Bujdosó and Hrotkó 2017). In temperate climates, sweet cherry production is one of the most rewarding sectors in fruit cultivation. In Bulgaria, sweet cherry is of significant economic importance and has a long-standing tradition of cultivation (Ser-

bezova 2019). As the first stone fruit to ripen, ahead of apricot, peach, and plum, sweet cherry is highly sought after in late spring and early summer (Bujdosó and Hrotkó 2017).

In recent decades, climate change, primarily driven by global warming, has caused significant economic harm in various regions worldwide. In Europe, sweet cherry cultivation can be severely affected by variations in winter chilling hours and spring warming hours, as well as by the frequency and severity of late spring frosts, rain, and hail during flowering and fruit. These factors, along with rainfall during the harvest period and elevated summer temperatures, pose significant challenges to cherry growers (Blanke et al. 2017).

Countries of the Balkan Peninsula have also been heavily affected by rising temperatures and changes in precipitation patterns, along with increasing frequency of extreme weather events, particularly droughts and frosts. In Bulgaria, recent climate trends have shown a deficit of atmospheric and soil moisture during critical periods of crop growth and yield formation (Georgieva et al. 2022). Nevertheless, Bulgaria offers excellent opportunities for growing stone fruit species, due to its favourable soil and climatic conditions characteristic of the temperate climate zone (Kazandjiev and Malasheva 2016).

Phenological observations play a vital role in understanding how fruit trees respond to environmental conditions. Climate is a significant limiting factor for sweet cherry production (Webster and Loney 1996). The microclimate of a particular habitat is shaped by variations in light, temperature, wind, and humidity, which collectively determine the feasibility of sweet cherry cultivation in that area. In recent decades, global climate change

has led to considerable alterations in these microclimatic conditions. Therefore, it is crucial to consider these changes to provide the successful growth of sweet cherry in the future (Blanke et al. 2017).

Understanding the flowering phenology of fruit species in temperate climates is becoming increasingly critical and is the focus of global research efforts (Legave et al. 2013, Chuine et al. 2016, Wenden et al. 2016, Darbyshire et al. 2017). The phenological traits of sweet cherry trees, including flowering and harvest dates, are influenced by a combination of factors, such as cultivar and rootstock choice, soil type, and the specific agro-ecological conditions in which they are grown (Jukić et al. 2020).

In temperate climates, the phenology of fruit trees is significantly influenced by seasonal variations in temperature. During the fall and winter months, these trees enter a state of dormancy, subsequently resuming active growth in spring. They then progress through a rapid series of developmental stages, ultimately reaching the flowering phase few weeks later (Rohde and Bhalerao 2007).

Understanding the phenological characteristics of different cultivars is crucial for making informed decisions regarding cultivar choice (Lisandru et al. 2017).

Therefore, this study was undertaken primarily to explore the phenological stages of development and the duration of the growing season for six sweet cherry cultivars grafted onto Maxma 14 rootstock. Additionally, it aimed to assess their adaptability to climatic conditions of the Plovdiv region.

Materials and Methods

The study was conducted in an experimental sweet cherry orchard at the Fruit

Growing Institute in Plovdiv from 2023 to 2024. This research focused on six sweet cherry cultivars: ‘Ferrovia’, ‘Regina’, ‘Kordia’, ‘Skeena’, ‘Lapins’, and ‘Canada Giant’, all grafted onto Maxma 14 rootstock (*Prunus mahaleb* × *Prunus avium*). The orchard was established in the spring of 2014, with trees planted at spacing 4.5 × 3.0 m. Throughout the experimental period (2023–2024), the sweet cherry cultivars were grown under irrigated conditions. The cultivar ‘Van’, grafted onto Mahaleb (*Prunus mahaleb* L.) rootstock, was used as standard for comparison.

Phenological phases were recorded according to Bleiholder et al. (2001). Observations were conducted every 1 to 2 days through direct visual monitoring in the orchard. The phenological data included the calendar dates of several main phenological stages:

- Bud swelling (BBCH 51);

- Flowering – beginning (BBCH 61), full (BBCH 65), and end (BBCH 69);
- Harvest maturity (BBCH 87);
- Length of the growing season – calculated in days from bud swelling to the end of leaf fall.

Temperature data were obtained from automated climate monitoring equipment, Meteobot Pro (Prointegra Ltd., Varna, Bulgaria), installed in the orchard.

Results and Discussion

In Plovdiv region, the average annual temperatures in 2024 increased by 0.5 °C compared to the previous year (Fig. 1). Ivanov (2022) noted a trend of rising average annual temperatures for this area. The most notable temperature deviations were recorded in February, April, and June, with increases of 3 °C, 4 °C, and

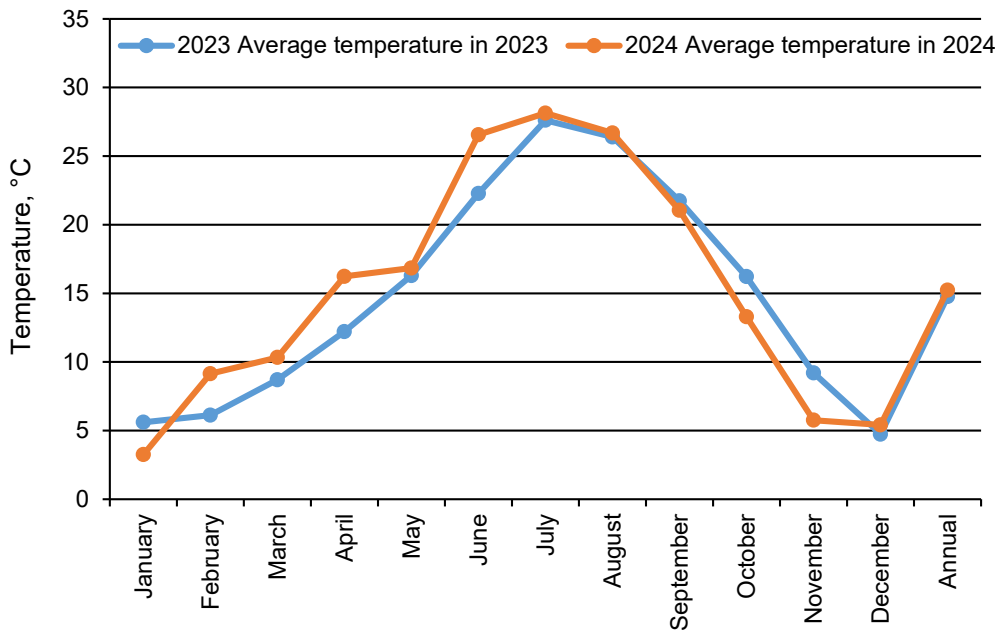


Fig. 1. Average monthly temperatures for 2023–2024.

4.3 °C, respectively. In March 2024, the average monthly temperature was 1.6 °C higher than in March 2023. This rise in temperature may contribute to an earlier onset of phenological phases and a delay in their progression (Shi et al. 2017).

Comparative analysis of average maximum and minimum temperatures from February to August in 2023 and 2024 indicates that temperatures were significantly lower in 2023 (Table 1). The most pronounced deviations were observed in April and June, with maximum temperature differences of 4.2 °C and 4.3 °C, respectively, and minimum temperature differences of 3.8 °C and 4.2 °C, respectively. The elevated temperatures recorded in February, March, and April 2024 noticeably influenced the phenological development of sweet cherry trees that year. Furthermore, lower values for both temperature indicators were recorded in January, which affected the accumulation of temperature sums essential for deep dormancy in 2024.

The beginning of active vegetation in fruit plants is characterised by the average

daily air temperature consistently exceeding 5 °C in spring (Vandova et al. 1984). In the Plovdiv region of Bulgaria, research on the agroclimatic conditions for cultivating various fruit species has revealed that the average air temperature consistently surpasses 5 °C at the beginning of March. The interval between the earliest and latest occurrences of this phenomenon can vary up to 42 days (Vandova et al. 1984). Full bud swelling typically occurs when the average daily temperature exceeds 5 °C, which is a critical indicator signalling the end of 'forced dormancy' in fruit species (Vandova and Filipov 1993, Vandova 2003).

In 2023, the initiation of vegetation, characterised by 'bud swelling' (BBCH 51), was observed in the studied cultivars between 17 and 27 February. The earliest occurrence was recorded in the cultivar 'Lapins', while the 'Canada Giant' and the standard cultivar 'Van' exhibited the latest bud swelling (Fig. 2). Notably, 'Kordia' displayed bud swelling 8 days earlier than the standard cultivar, whereas 'Ferrovia' and 'Skeena' began this phase 7 days

Table 1. Comparative analysis of monthly temperature variations between 2023 and 2024.

Month	Maximum temperature in 2023	Maximum temperature in 2024	Variation	Minimum temperature in 2023	Minimum temperature in 2024	Variation
January	6.1	3.8	2.3	5.1	2.7	2.4
February	6.9	9.8	-2.9	5.4	8.5	-3.1
March	9.4	10.9	-1.5	8.0	9.8	-1.8
April	12.9	17.1	-4.2	11.6	15.4	-3.8
May	16.9	17.4	-0.5	15.7	16.3	-0.6
June	23.0	27.4	-4.4	21.5	25.7	-4.2
July	28.5	29.0	-0.5	26.7	27.3	-0.6
August	27.2	27.6	-0.4	25.6	25.8	-0.2
September	22.5	21.8	0.7	21.0	20.3	0.7
October	17.0	14.2	2.8	15.4	12.4	3.0
November	9.8	6.4	3.4	8.6	5.1	3.5
December	5.3	5.8	-0.5	4.2	5.1	-0.9
Annual	15.5	15.9	-0.5	14.1	14.5	-0.5

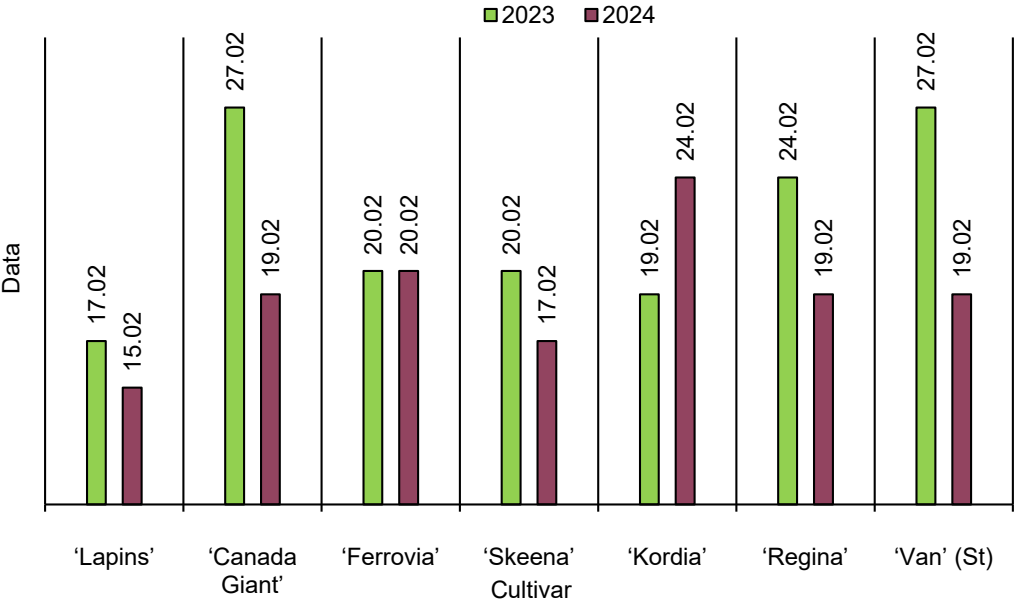


Fig. 2. Onset of vegetation (BBCH 51) in sweet cherry cultivars during the 2023–2024 period.

earlier than ‘Van’.

In the second year, most of the studied cultivars showed an earlier onset of vegetation, occurring 2 to 8 days sooner than in the previous year. The cultivar ‘Kordia’ was an exception, experiencing a 5-day delay, while ‘Ferrovia’ displayed bud swelling on the same date (20 February) in both years.

The onset of flowering in this fruit species is influenced by the cultivars and the climatic conditions of the growing region, and it can vary from year to year. Flowering begins when the average daily air temperature consistently exceeds 8.4 °C, which is considered the biological minimum for floral induction in this species (Khodorova and Boitel-Conti 2013).

The earliest onset of flowering (BBCH 61) was observed in the cultivar ‘Lapins’ in both years, followed closely by the standard cultivar ‘Van’, with flowering com-

mencing at the end of March (Fig. 3). For all other sweet cherry cultivars, the BBCH 61 phase was recorded between 3 and 5 April in 2023 and between 1 and 3 April in 2024. Notably, the cultivar ‘Skeena’ also began flowering at the end of March in 2024, three days earlier than in the previous year.

Full flowering (BBCH 65) was first observed in the cultivar ‘Lapins’ occurring on 29 March in both 2023 and 2024, which was seven days earlier than in ‘Van’ in the first year and two days earlier in the second. For all other cultivars, full flowering took place 2 to 8 days after the standard cultivar ‘Van’ and 9 to 15 days later than ‘Lapins’. In 2024, a notable shift in flowering onset was evident across all sweet cherry cultivars (except ‘Lapins’), with full flowering occurring 4 to 8 days earlier than in the previous year. Additionally, the duration of the BBCH 65 phase was 2 to 5

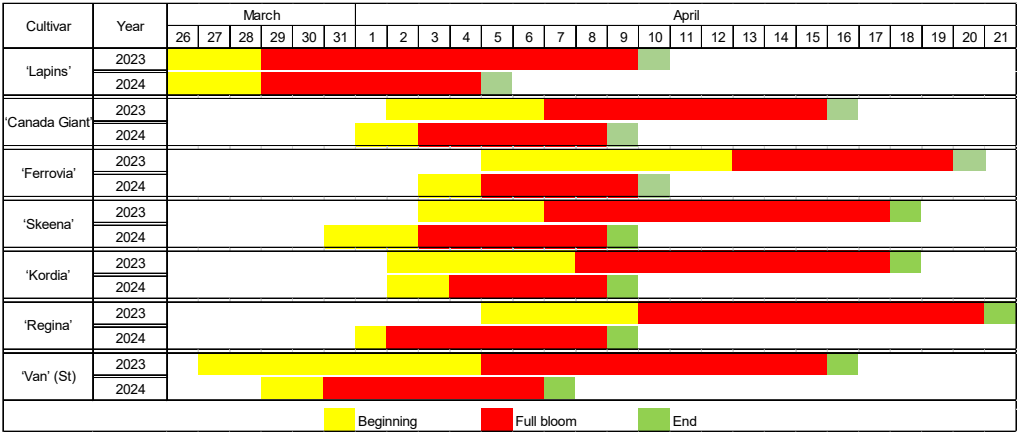


Fig. 3. Phenological diagram of the flowering stages in sweet cherry cultivars during the 2023–2024.

days shorter compared to 2023.

The flowering period (from start to finish) in 2023 lasted between 15 and 21 days, with the cultivar 'Canada Giant' having the shortest duration and the standard cultivar 'Van' having the longest. In 2024, the flowering period was reduced by 7 to 10 days, with the longest duration recorded in 'Lapins' (11 days), followed by 'Van' and 'Skeena' (10 days). The shortest flowering periods were observed in the cultivars 'Ferrovia' and 'Kordia,' each lasting 8 days.

The phenology of fruit trees, particularly flowering, is strongly influenced by temperature changes. Drkenda et al. (2018) reported a rising trend in air temperatures, which has led to earlier onset of the flowering phase.

In 2023, the earliest harvest maturity was observed in 'Canada Giant' and the standard cultivar 'Van', which reached this stage during the first ten days of June. In contrast, the latest harvest maturity was observed in 'Lapins' on 20 June (Table 2). For all other sweet cherry cultivars, harvest maturity occurred in the last ten days of June, resulting in a delay of 4 to 7 days compared to the standard cultivar.

The period from full flowering (BBCH 65) to harvest maturity (BBCH 87) during the first experimental year ranged from 62 to 84 days. In the second year, most evaluated cultivars reached the BBCH 87 phase 3 to 7 days earlier than in 2023. However, the cultivars 'Ferrovia' and 'Skeena' experienced delays in reaching the BBCH 87 phase, occurring 7 and 4 days later, respectively, in 2024.

Throughout the reporting period, variations in the duration of the growing season were observed for all analysed sweet cherry cultivars (Fig. 4). The most pronounced deviations were identified in the cultivars 'Van' and 'Regina', with growing seasons in 2024 being 20 days and 13 days shorter, respectively, compared to 2023. Conversely, the cultivar 'Lapins' displayed the smallest change, as its growing season ended only 2 days earlier in the second reporting period. Notably, the cultivar 'Canada Giant' experienced a longer growing season in 2024, extending 4 days beyond that of 2023.

Chmielewski and Rötzer (2001) found that a 1 °C increase in temperature from February to April, above the average,

Table 2. Duration of the period from full flowering to harvest ripeness in sweet cherry cultivars during the 2023-2024 period.

Cultivar	Year	BBCH 65	BBCH 87	From BBCH 65 to BBCH 87, days
'Lapins'	2023	29 March	20 June	84
	2024	29 March	13 June	77
'Canada Giant'	2023	7 April	7 June	62
	2024	3 April	31 May	59
'Ferrovia'	2023	13 April	15 June	64
	2024	5 April	14 June	71
'Skeena'	2023	7 April	12 June	67
	2024	3 April	12 June	71
'Kordia'	2023	8 April	15 June	69
	2024	4 April	6 June	64
'Regina'	2023	10 April	15 June	67
	2024	2 April	7 June	67
'Van' (St)	2023	5 April	8 June	65
	2024	31 March	30 May	61

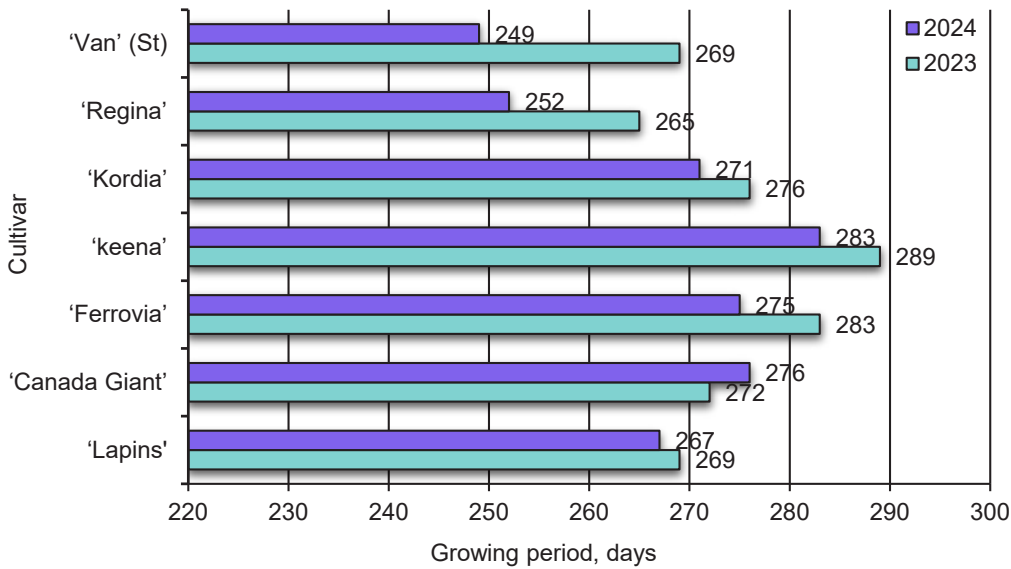


Fig. 4. Vegetation period in sweet cherry cultivars during the 2023-2024 period.

causes beginning of the growing season approximately 7 days earlier. Furthermore, a trend toward an extended growing sea-

son has been observed, with a 1 °C rise in average annual air temperature resulting in a 5-day increase in its length.

Conclusions

This study demonstrated that temperature fluctuations had a significant impact on the onset of vegetation, particularly during the 'bud swelling' phase (BBCH 51), across various sweet cherry cultivars. Notably, the cultivar 'Lapins' exhibited the most pronounced differences, displaying the earliest onset of growing in both years examined. Additionally, the BBCH 65 phase in 'Lapins' occurred earlier than in the other sweet cherry cultivars analysed. Interestingly, despite the temperature variations observed during the study years, the date of full flowering remained consistently unchanged. However, the duration from full flowering to harvest maturity was the longest for the cultivar 'Lapins' throughout the entire reporting period.

In the cultivar 'Canada Giant', full flowering began 9 days later in 2023 and 5 days later in 2024 compared to 'Lapins', which typically started in the first ten days of April, like most other studied cultivars. However, the period from full flowering to harvest maturity (BBCH 87) was shortest in 'Canada Giant'.

In 2024, the expected increase in temperatures was anticipated to have a significant impact on the duration of the growing season. Notably, significant changes were identified in the cultivars 'Van' and 'Regina', with their growing seasons ending 20 and 13 days earlier, respectively, in the second reporting year.

Alterations in the timing of phenological phases in sweet cherry trees can have significant economic implications, directly affecting both yield development and overall production. This study revealed that temperature fluctuations play a significant role in determining the onset of the growing season, as well as the duration of flowering and growth periods in sweet

cherry trees. The findings underscore the need for more comprehensive research and the formulation of phenological models to assess the effects of climate change on sweet cherry cultivation in Southern Bulgaria.

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Subalpine willow shrub vegetation on Vitosha Mountain

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Thematic field: Ecosystem biodiversity, conservation and environmental restoration

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Abstract

Subalpine willow shrublands are locally distributed in Vitosha Mt. and are dominated by low-growing willows (*Salix lapponum* L., *Salix waldsteiniana* Willd.). During the 2021–2022 field seasons, 21 relevés were collected following the Braun-Blanquet approach. Complete information on the species composition and abiotic characteristics of the environment (elevation, aspect, slope, soil depth) in all sample plots was collected. Diagnostic, constant and dominant species were identified for all established syntaxa. The aims of this study are: (1) to establish the syntaxonomical diversity of subalpine willow shrub vegetation on Vitosha Mt., Bulgaria; and (2) to reveal the species composition and vegetation structure of the established syntaxa. For the first time, the distribution of 2 associations representing subalpine willow shrublands has been established for the vegetation of the country – *Salicetum lapponum* Zlatník 1928 and *Salicetum waldsteinianae* Beger ex Oberdorfer 1978. Association *Salicetum lapponum* is dominated by the downy willow *Salix lapponum*. It reaches 1–1.5 m height and covers 85–100 %. Association *Salicetum waldsteinianae* is dominated by *Salix waldsteiniana*, which covers 95–100 %. From ecological point of view, ass. *Salicetum lapponum* occupied wetter habitats with acidic soil reaction and lower nutrient content, whereas ass. *Salicetum waldsteinianae* is found in less acidic microhabitats, with higher nutrient content. Habitats of both willows are listed in the Habitat Directive 92/43/EEC (4080 Sub-Arctic *Salix* spp. scrub), Annex I of the Bulgarian Biodiversity Law and the Red Data Book of the Republic of Bulgaria. Main pressures and threats affecting those phytocoenoses are climate change and especially changes in precipitation regimes, natural succession and the influence of human activities.

Key words: habitats, Natura 2000, vegetation classification, *Salix* communities.

Introduction

Mountain ecosystems are very important, not just for the rich biodiversity they support, but also for their crucial roles in providing many ecosystem services, such as regulating climate and managing water cycles (Glushkova et al. 2020). It's noteworthy that almost half of the world's biodiversity hotspots are found in mountainous regions, emphasizing their great conservation value (Barthlott et al. 2005, Millennium Ecosystem Assessment 2005, Mutke and Barthlott 2005).

Arctic-alpine vegetation includes both shrub and dwarf shrub communities, as well as grasslands. This vegetation is presented in Bulgaria mainly by the phytocoenoses of *Juniperus communis* subsp. *nana* Syme, *Rhododendron myrtifolium* Schott & Kotschy, *Vaccinium* spp., *Salix reticulata* L., *Dryas octopetala* L., *Bruckenthalia spiculifolia* (Salisb.) Rchb., *Nardus stricta* L., *Festuca airoides* Lam., *Juncus trifidus* L. and typically occurs above 2000 m a.s.l. with the largest areas represented in Rila and Pirin Mts (Velchev 2002).

Salix waldsteiniana Willd. is Alpo-Carpato-Balcanic floristic element and occurs in Bulgaria in Central Stara Planina, Vitosha, Rila, Pirin and West Frontier Mountains floristic regions at altitudes 1500–2500 m a.s.l., while *Salix lapponum* L. is Euro-Siberian floristic element and found in Vitosha and Rila floristic regions only within the range 1500–2600 m a.s.l. (Assyov and Petrova 2012). The phytocoenoses of *S. waldsteiniana* and *S. lapponum* are defined as 'Rare plant communities' on the territory of Bulgaria (Velchev and Bondev 1984). Despite their relatively limited distribution in the country, both species

are not considered endangered (Petrova and Vladimirov 2009) and are glacial relict species. These two subalpine willows are dominants and diagnostic species for habitat 4080 Sub-arctic *Salix* spp. scrub of the Habitat Directive 92/43/EEC, occurring in Bulgaria only in Vitosha and Rila Mts (Kavrakova et al. 2009) and protected by the Natura 2000 ecological network.

There are different opinions in classifying these willow stands, as they were related to two alliances of two different classes – in *Adenostylion alliariae* Br.-Bl. 1926 alliance of the *Mulgedio-Aconitetea* Hadač et Klika in Klika et Hadač 1944 class (Kočí 2007) or *Alnion viridis* Schnyder 1930 alliance of the *Betulo carpaticae-Alnetea viridis* Rejmánek ex Boeuf, Theurillat, Willner, Mucina et Simler in Boeuf et al. 2014 class (Willner and Grabherr 2007, Mucina et al. 2016, Dakskobler and Surina 2017). In Bulgaria willow vegetation above the treeline is poorly studied. Mucina et al. (1990) described ass. *Bartsio-Salicetum reticulatae* Mucina et al. 1990 from Pirin Mt. Limited data have been published from Rila Mt. (Roussakova 2000), where the phytocoenoses of *Salix herbacea* L. have been studied (ass. *Primulo-Salicetum herbaceae* Tzonev et al. 2009), as well as *Salix retusa* L. and *S. reticulata* communities (ass. *Salicetum retusae-reticulatae* Braun-Blanq. 1926) (Tzonev et al. 2009). These associations are related to *Salicion herbaceae* Br.-Bl. 1926 alliance of the *Salicetea herbaceae* Br.-Bl. 1947 class (Tzonev et al. 2009).

The aims of this study are: (1) to establish the syntaxonomical diversity of subalpine willow shrub vegetation on Vitosha Mt., Bulgaria; and (2) to reveal the species composition and vegetation structure of the established syntaxa.

Material and Methods

Study area

The study was conducted on Vitosha Mt., Bulgaria (Fig. 1). All collected vegetation plots fall within the protected site SCI BG0000113 'Vitosha'. The studied area is located in the subalpine belt at elevations 1800–2040 m a.s.l. The highest mountain peak is Cherni vrah (2290 m). Many rivers spring and form peat bogs in this area and do not dry up even in the hottest summer months. Vitosha Mt. is built of a Syenite core covered with volcanic rocks, such as Andesites, Diorites, and other rock types (Nikolov and Jordanova 2002). The mountain is characterised by a diverse climate. It is defined as Continental with high amplitude between summer and winter temperatures and maximum precipita-

tion in May–June. In the range from 1400 to 1900 m a.s.l., the winter endures ca. five months with snow cover and the average annual air temperature measured at 1840 m is 3.3° C (Nikolov and Jordanova 2002).

Data sampling and analysis

During the 2021–2022 field seasons, 21 vegetation plots (relevés) were established from the subalpine zone of Vitosha Mt. (Fig. 1), following the Braun-Blanquet approach (Westhoff and van der Maarel 1973). The plot size was 60–78 m² as recommended for shrubland vegetation (Chytrý and Otýpková 2003). All relevés were stored in the Balkan Vegetation Database (Vassilev et al. 2020, currently including 29,900 relevés). For each relevé, full spe-

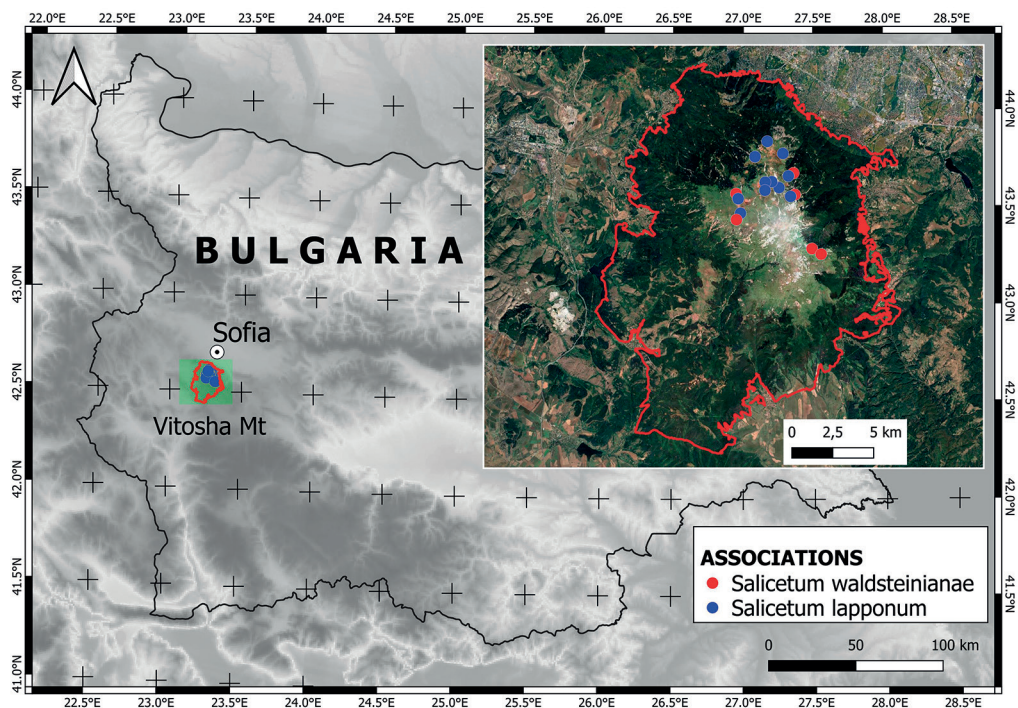


Fig. 1. Map of the study area showing sampled vegetation locations.

cies composition and layer structure were recorded. Additionally, information about abiotic characteristics of the environment (elevation, aspect, slope, soil depth, and bedrock type) was collected.

Elevation, slope inclination, and location were determined using a Garmin eTrex Vista device, and the aspect of the slope was measured with a compass for each relevé. Given the relatively poor development of the soil cover in the study area, we estimated soil depth by three conditional categories for the purposes of this study: (1) shallow (<10 cm depth), (2) moderately deep (10–20 cm), and (3) deep (>20 cm).

Classification of relevés was carried out using JUICE 7.0 software (Tichý 2002). The numerical classification was performed with PC-ORD (McCune and Mefford 1999) and JUICE 7.0 (Tichý 2002) software packages. Sørensen (Bray-Curtis) was used as a distance measure and similarity was calculated using Ward's clustering method.

The diagnostic species were identified according to the Phi-coefficient (Chytrý et al. 2002). Only statistically significant Phi-coefficients values, evaluated using Fisher's exact test ($P < 0.05$), are presented in the analytical table (Appendix A, Table 1). All clusters were standardised to equal sizes. The threshold value for a species that is considered as diagnostic was set at a Phi-coefficient ≥ 0.3 (multiplied by 100). Species with Phi-coefficient ≥ 0.5 have been considered highly diagnostic. The species in the analytical table have been ordered by decreasing fidelity values. The diagnostic role of the species has also been considered according to available literature sources.

The constancy of the species was presented by percentages in the analytical table. Species with cover above 50 % in

at least 5 % of the relevés in any cluster were considered as dominants, whereas constant species were those having at least 50 % presence in a cluster.

Detrended correspondence analysis (DCA) was applied as an indirect ordination technique using the CANOCO 4.5 software package (ter Braak and Šmilauer 2002) to reveal the major environmental gradients shaping the floristic composition. Square root transformation and down-weighting of rare species have been applied. The habitat's ecological conditions were assessed using the 'Ellenberg indicator values' (Ellenberg 1979, Ellenberg et al. 1992) and projected onto the ordination space.

The nomenclature of the vascular plants follows Stoyanov et al. (2022) and was subsequently standardised according to the Euro+Med PlantBase (Euro+Med 2025). The floristic elements were interpreted according to Assyov and Petrova (2012) and the species life forms were determined according to Dřevojan et al. (2023). The assessment of present floristic elements and life forms in the established phytocoenoses was assessed as species richness and cover/abundance of each group.

The nomenclature of the higher-rank syntaxa was harmonised with Mucina et al. (2016). In addition, bryophytes and lichens were excluded from the species composition because they were still not identified for all relevés.

Results and Discussion

The subalpine willow shrublands on Vitosha Mt. were related to 1 class, 1 order, 1 alliance, and 2 associations. The proposed syntaxonomical scheme is:

Class ***Betulo carpaticae-Alnetea***

viridis

Order ***Alnetalia viridis*** Rubel ex Karner et Willner in Willner et Grabherr 2007

Alliance ***Alnion viridis***

Ass. ***Salicetum waldsteinianae*** Beger ex Oberdorfer 1978*

Ass. ***Salicetum lapponum*** Zlatník 1928*

The new syntaxa for the vegetation of Bulgaria are marked with *.

Ass. *Salicetum waldsteinianae* * (Appendix A, Table A1, rel. 1 – 6; Fig. 2, Cluster 1)

Diagnostic species: *Salix waldsteiniana*

Constant species: *Rubus idaeus* L., *Calamagrostis arundinacea* (L.) Roth

Dominant species: *Salix waldsteiniana*, *Aegopodium podagraria* L.

Distribution and ecology: These stands occur in the subalpine part of Vitosha Mountain on even or slightly inclined terrains (2–3 °) regardless of aspect. They

are found in natural depressions in the terrain where the soil is not eroded and cover a limited area of 60–120 (150) m². The terrains are mostly rocky. The soils are predominantly moderately deep and rarely shallow. Typically, the subalpine zone of Vitosha Mt. is characterised by a short growing season and abundant rainfall. In recent years, periods of drought also occur (for example, during 2024), which has a negative effect on this vegetation type. The stands are located within the borders of the Vitosha Nature Park and the two reserves, Bistrishko Branishte and Torfeno Branishte. There, they are under regulative measures for protection and grazing is absent, which leads to the spread of the *Salix waldsteiniana* shrubs in the neighbouring habitats, too. The species is also found as singular shrubs within the phytocoenoses of *Juniperus communis* subsp. *nana*, *Vaccinium myrtillus* L., *V. vitis-idaea* L., *Nardus stricta*, *Festuca valida* (Velen.) Péntzes. In some places it occurs togeth-

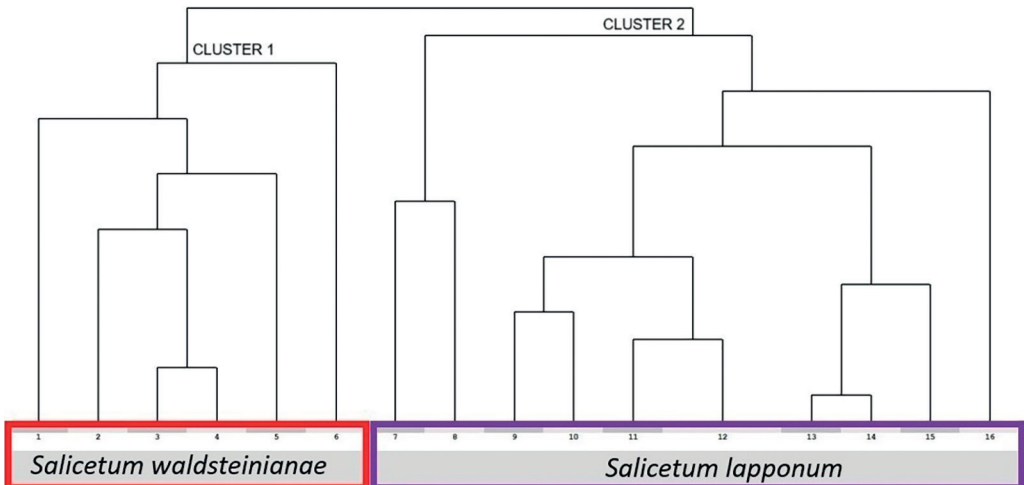


Fig. 2. Classification dendrogram of the analysed data set with cluster interpretation.

Note: According to PC-ORD classification, two well-distinguished associations of *Betulo carpaticae-Alnetea viridis* class are identified. Associations established indicated on the X-axis. Cluster similarity expressed on the Y-axis.

er with *Salix lapponium*, *S. caprea* L., and *Rubus idaeus*.

The analysis of the life-form composition in the phytocoenoses shows predominance of hemicryptophyte (54 % of all species), followed by phanerophytes (31 %). On the other hand, based on cover/abundance species data phanerophytes dominated in the species composition with 91 %, whereas hemicryptophytes are represented by only 9 %. SubBoreal (31 % of all species), Boreal (17 %) and Alpo-Carpato-Balcanic (11 %) floristic elements dominated in the species composition. The comparison of the species cover/abundance shows the prevalence of Alpo-Carpato-Balcanic elements (88 %), followed by subBoreal (14 %) and Euro-Siberian elements (11 %).

Vegetation description and syntaxonomy: The stands of the association are characterised by closed horizontal structure with a total cover of 95–100 % and a well developed herb layer in 3 of the plots, where the level of shading allows to be formed between the shrubs of *Salix waldsteiniana* and has a cover of 20–75 %. In the other 3 plots, the herb layer covered between 2 and 8 %. The bryophytes and lichens are either missing or have a cover of less than 1 %. A considerable amount of litter accumulated during the vegetation seasons, covering 30–65 % of the surface.

The stands are characterised by a poor species composition with the number of species varying between 6 and 20 (11 on average). The dominant species is *Salix waldsteiniana*. Species widespread in the neighbouring grassland and shrubland habitats also occur in these phytocoenoses. Such are the diagnostic species for the classes *Mulgedio-Aconitetea* (*Calamagrostis arundinacea*, *Cirsium appendiculatum* Griseb., *Doronicum austri-*

acum Jacq., *Geum coccineum* Sibth. & Sm., *Luzula luzuloides* (Lam.) Dandy & Wilmott), *Epilobietea angustifolii* Tüxen et Preising ex von Rochow 1951 (*Aegopodium podagraria*), *Scheuchzerio palustris-Caricetea fuscae* Tüxen 1937 (*Carex nigra* (L.) Reichard), *Juncetea trifidi* Hadač in Klika et Hadač 1944 (*Festuca valida*), *Loiseleurio procumbentis-Vaccinietea* Eggler ex Schubert 1960 (*Juniperus communis* subsp. *nana*) and *Robinietae* Jurko ex Hadač et Sofron 1980 (*Rubus idaeus*, *Salix caprea*).

Association *Salicetum waldsteinianae* is also spread in Austria (Karner 2007a, b) and Slovenia (Zupančič and Žagar 2001, Dakskobler and Surina 2017). Zupančič and Žagar (2001) record var. geogr. *Homogyne sylvestris* for Slovenia. Three subassociations are given for Austria (*typicum*, *rhododendretosum ferruginei* and *aceretosum pseudoplatani*). In regard to the species composition, the stands of the association in Bulgaria differ from those in Austria and Slovenia. The domination of *Salix waldsteiniana* is a common characteristic, but the accompanying species are different to some extent. A characteristic species for association in Slovenia are also *Salix glabra* Scop., which is not distributed in Bulgaria. Also, differential species of the geographic variant are *Homogyne sylvestris* Cass., *Knautia drymeia* Heuff. subsp. *drymeia*, *Aposeris foetida* (L.) Less. and *Galium laevigatum* L. The variance in the species composition uncovers the local endemism of the phytocoenoses of the *Salicetum waldsteinianae* association in different localities.

Within the territory of Bulgaria, *Salix waldsteiniana* is also found in Stara planina, Rila, Pirin, and Western Border Mts (Assyov and Petrova 2012). According to Bondev et al. (1982), the formation *Saliceta waldsteiniana* was recorded in Torfeno

Branishte Reserve on Vitosha Mountain, following the dominance approach. Additional research will be necessary to expand the knowledge about the ecology and species composition of the *Salicetum waldsteinianae* association across the entire territory of the country.

Ass. *Salicetum lapponum* * (Appendix A, Table A1, rel. 7–17; Fig 2, Cluster 2)

Diagnostic species: *Salix lapponum*, *Potentilla erecta*, *Deschampsia caespitosa*

Constant species: *Geum coccineum*, *Carex nigra*, *Senecio nemorensis* L.

Dominant species: *Salix lapponum*

Distribution and ecology: The association is more widespread in the subalpine part of Vitosha Mountain between 1800 and 1966 m a.s.l. It occurs on flat terrains in natural depressions, around springs or places with high levels of underground water. Unlike the stands of the association *Salicetum waldsteinianae*, its habitats are more waterlogged and swampy. The stands occupy limited areas of 50–150 (170) m². Individual shrubs of *Salix lapponum* are spread to the neighbouring phytocoenoses, too, and, as a whole, the association has a stable distribution with a tendency to expand its territory. The soils are moderately deep and often are over-moisturised. The main unfavourable factors are climate change, leading to less precipitations and prolonged periods of drought during the summer months.

Vegetation description and syntaxonomy: The phytocoenoses are characterised by a closed horizontal structure and low number of vascular plants (the average species number is 12). *Salix lapponum* is a monodominant species with a total cover of 100 %. The shrub layer has a cover of 75–100 % (91 % on average). Apart from *Salix lapponum*, other shrub species that are found in the phy-

tocoenoses are *Salix caprea*, *Vaccinium myrtillus*, and *Juniperus communis* subsp. *nana*. The herb layer has a total cover of 7 to 70 % (43 % on average). The species with higher cover and abundance are *Carex nigra*, *Deschampsia cespitosa* (L.) P. Beauv., *Geum coccineum*, *Senecio nemorensis*, *Molinia coerulea* (L.) Moench. Bryophytes mostly cover up to 2–3 % of the plots, but in one of the sample plots, their cover is 20 %. Litter cover is 10–40 %.

The spread of the association in waterlogged microhabitats predetermines the spread of some hygrophyllic species typical for the phytocoenoses of the class *Scheuchzerio palustris-Caricetea fuscae*, which occur in their vicinity (such as *Carex nigra*, *Geum coccineum*, *Molinia coerulea*, *Deschampsia cespitosa*, *Potentilla erecta* (L.) Raeusch.). Other species with more significant abundance and coverage are the moesophyllic species *Agrostis capillaris* L., and *Calamagrostis arundinaceae*, which occur in the neighbouring grassland phytocoenoses.

The analysis of the life forms shows predominance of hemicryptophytes (70 % of all species), followed by phanerophytes (17 %). On the other hand, based on cover/abundance species data, phanerophytes dominated in the species composition with 89 %, whereas hemicryptophytes were represented by only 19 %. Boreal (35 % of all species), subBoreal (30 %), Euro-Siberian (11 %) and Alpo-Carpatic (6 %) floristic elements dominated in the species composition. The comparison of the species cover/abundance shows a prevalence of Euro-Siberian elements (88 %), followed by Boreal (15 %), Alpo-Carpatic (12 %) and subBoreal (9 %) elements.

Bondev et al. (1982) record the formation *Saliceta lapponii* according to the

dominance approach and 2 associations – *Salix lapponum-Deschampsia caespitosa* and *Salix lapponum-Molinia coerulea* – within the Torfeno Branishte Reserve on Vitosha Mountain. It is noted that *Salix lapponum* forms monodominant phytocoenoses or in complexes with the coenoses of other peatland species.

The association *Salicetum lapponum* was described from Krkonoše Mts in the Czech Republic by Zlatník in 1928. According to Kočí (2007), this association represents a glacial relict of arctic origin in the Krkonoše and Hrubý Jeseník Mountains. It is also recorded for Poland by Matuszkiewicz and Matuszkiewicz (1975). Biosystematic and ecological investigation of *Salix lapponum* in Central Europe was done by Hroneš (2018).

Syntaxonomical scheme

Some authors classify the high-mountain phytocoenoses of the two willow species to the *Adenostylion alliariae* alliance (Kočí 2007, Hroneš 2018), while others consid-

er them to belong to the *Alnion viridis* alliance (Župančič and Žagar 2001; Karner 2007a, 2007b; Dakskobler and Surina 2017). According to Mucina et al. (2016), the alliance *Alnion viridis* includes subalpine green alder scrub on fertile soils of the Alps and the Balkans and belongs to the class *Betulo carpaticae-Alnetea viridis* where subalpine willow shrubs are classified, as well.

Vegetation – environment relationships

The first axis is related to the continentality, nutrient content and soil reaction conditions (figs 3 and 4). The eigenvalue of the first axis (0.473) exhibits a well-distributed pattern of the sample plots along it. Its gradient length is 3.153 and explains 16.96 % of the total inertia (2.789) of the data set. Stands of association *Salicetum lapponum* occupied wetter microhabitats with more acidic soil reaction and lower nutrient contents. In contrast, ass. *Salicetum waldsteinianae* is distributed in less

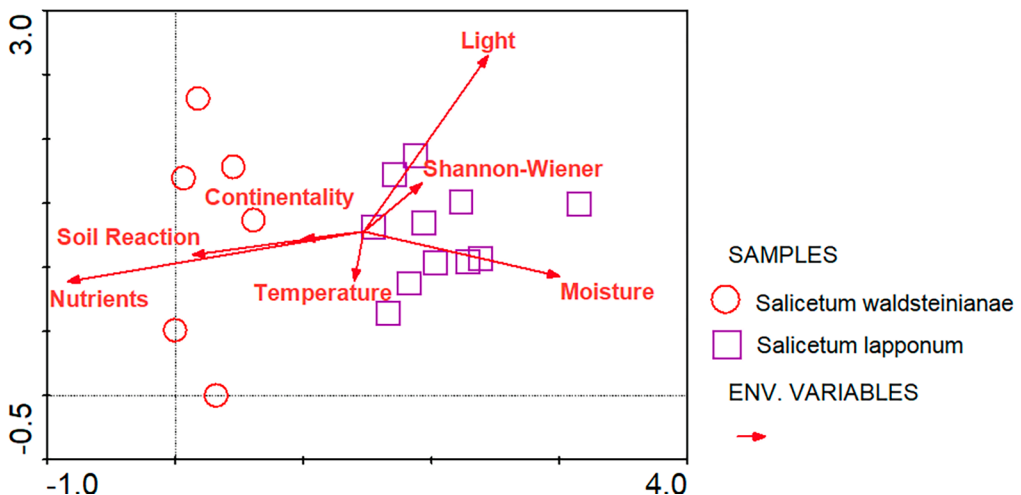


Fig. 3. Ordination diagram of the analysed data set along the first two axes.

Note: Variables (EIV) are passively projected onto the ordination space.



Fig. 4. Communities of *Salix lapponum* and *S. waldsteiniana* in subalpine belt of Vitosha Mt.

acidic microhabitats with higher nutrient content. The variability expressed by the second axis represented the temperature and light. The gradient length of the second axis is 2.317, standard deviation units, indicating a moderate ecological gradient. The variance explained by the second DCA axis (0.243) accounts for 8.71 % of the total inertia in the data set. The stands of ass. *Salicetum lapponum* have a little bit less cover of shrub layer, whereas communities of ass. *Salicetum waldsteinianae* have a stronger shading effect.

Conservation status and protection

Species *Salix waldsteiniana* and *S. lapponum* are not included in the Red List of Bulgarian vascular plants (Petrova and Vladimirov 2009) and the Red Data Book

of the Republic of Bulgaria, Vol. 1, Plants and Fungi (Peev 2015). The habitat they form is listed below the Habitat Directive 92/43/EEC (4080 Sub-Arctic *Salix* spp. scrub) and Annex I of the Bulgarian Biodiversity Law. The whole national area of the habitat (17.79 ha) is included in the ecological network Natura 2000 in Bulgaria. The habitat is presented in two protected sites – SCI BG0000113 ‘Vitosha’ and SCI BG0000496 ‘Rilski Manastir’. A significant part of the habitat area also falls within the ‘Torfeno Branishte’ and ‘Bistrishko Branishte’ reserves within SCI BG0000113 ‘Vitosha’. This habitat type is also included in the Red Data Book of the Republic of Bulgaria, Vol. 3, Natural habitats with code 14F2 Subalpine willow communities (*Salix* spp.) as ‘Endangered’ [EN – A1, 2 B1 C2 D2 E2 G2 H2 I1] (Rous-

sakova 2015). The habitat is included in the Management Plan of Vitosha Nature Park within the group of habitats defined as 'Habitats whose conservation within the territory of Vitosha Nature Park is of paramount national importance' and is included in the Site-Specific Conservation Objectives for SCI BG0000113 'Vitosha', declared by the Minister of Environment and Water.

Threats risk factors

The specific phytocoenoses of *Salix waldsteiniana* and *S. lapponum* are under many threats. Among the main identified pressures and threats are climate change and especially changes in precipitation regimes – climate aridification, natural succession, direct influence of human activities, such as sports, tourism and recreation, creation of infrastructure for sports, tourism, and leisure activities, etc.

Conclusions

The subalpine willow shrublands on Vitosha Mt. are related to one alliance and two associations – ass. *Salicetum waldsteinianae* and ass. *Salicetum lapponum*. Both associations are characterised by a closed horizontal structure with total vegetation cover reaching up to 100 %, and by species-poor composition (11–12 species on average). Stands of the association *Salicetum waldsteinianae* occur in the subalpine part of Vitosha Mountain on even or slightly inclined terrains of any aspect. This vegetation covers a limited area and is located in natural depressions in the terrain where the soil is not eroded. Stands of the association *Salicetum lapponum* are more widespread in the subalpine belt of Vitosha Mt., occupying

more waterlogged and swampy habitats, unlike those of the ass. *Salicetum waldsteinianae*.

Both associations represent a habitat type listed under the Habitat Directive 92/43/EEC (4080 Sub-Arctic *Salix* spp. scrub) and Annex I of the Bulgarian Biodiversity Law. This vegetation type is also included in the Red Data Book of the Republic of Bulgaria, Vol. 3, Natural habitats. This vegetation is sensitive regarding the presence of abundant moisture and is under many threats. Among the main identified pressures and threats are climate change and especially climate aridification.

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Table A1. Analytical table of subalpine willow vegetation on Vitosha Mt.

Floristic elements	Relevé number		30427	30429	32138	33317	33315	33312	30430	32140	30435	32184	32169	32191	33314	32166	33316	33313	32177											
	Table number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17											
	Balkan Vegetation Database number		30427	30429	32138	33317	33315	33312	30430	32140	30435	32184	32169	32191	33314	32166	33316	33313	32177											
	Date (year/month/day)		20220712	20220713	20220816	20210810	20210804	20210804	20220713	20220817	20220713	20220819	20220818	20220819	20210804	20220818	20210805	20210804	20220819											
	Relevé area, m²		64	64	64	60	60	64	64	64	64	64	64	64	64	64	78	64	64											
	Elevation, m		2041	1836	1880	1888	1976	1929	1840	1815	1945	1925	1811	1937	1981	1820	1800	1966	1929											
	Aspect, degrees		135			360	225									225														
	Slope, degrees		2			2	3									3														
	Cover total, %		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100											
	Cover shrub layer, %		100	95	95	100	100	100	75	85	85	95	100	90	100	95	100	100	85											
	Cover herb layer, %		75	15	8	2	6	20	75	60	70	30	7	50	12	7	10	10	40											
	Cover moss layer, %										2							20	2											
	Cover litter layer, %								40		30	20	10	10		30			10											
	Cover bare rock, %															5														
	Soil depth		2	2	1	2	2	2	2	2	2	2	2	2	2	1	2	2	2											
Number of species		11	12	7	7	6	20	12	16	14	18	11	9	8	12	8	12	9												
Diagnostic species of ass. <i>Salicetum waldsteinianae</i> Beger ex Oberdorfer 1978																														
Alp-Carp-Bal	Ph	4		5	5	5	4	5	3				2						83.2	100	18									
Diagnostic species of ass. <i>Salicetum lapponum</i> Zlatnik 1928																														

Eur-Sib	Ph	4	<i>Salix lapponum</i> L.	.	.	.	.	.	4	5	5	5	5	5	5	5	100	0	100
subBo- real	H	6	<i>Potentilla erecta</i> (L.) Raeusch.)	.	.	.	.	.	+	r	r	r	r	.	.	r	75.6	0	73
Boreal	H	6	<i>Deschampsia caespitosa</i> (L.) P. Beauv	.	.	.	.	.	+	+	1	+	2	r	.	r	1	+	2
Diagnostic species of all. <i>Alnion viridis</i> Schnyder 1930, ord. <i>Alnetalia viridis</i> Rubel ex Karner et Willner in Willner et Grabherr 2007 and cl. <i>Betulo carpaticae-Alnetea viridis</i> Rejmánek ex Boeuf, Theurillat et al. in Boeuf et al. 2014 class																			
subBo- real	H	6	<i>Calamagrostis arundina- cea</i> (L.) Roth	1	+	+	.	.	r	2	2	.	+	.	r	.	.	.	67
Boreal	H	6	<i>Hypericum maculatum</i> Cranz.	.	.	.	r	.	r	.	r	.	.	.	.	.	r	.	33
Eur	H	6	<i>Luzula sylvatica</i> (Huds.) Gaudin	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	17
Boreal	H	6	<i>Senecio nemorensis</i> L.	1	.	+	r	.	.	.	r	r	2	.	r	2	.	.	50
Diagnostic species of cl. <i>Mulgedio-Aconitetea</i> Hadač et Klika in Klika et Hadač 1944																			
Eur-As	G-H	6	<i>Veratrum lobellianum</i> Bernh.	r	r	.	.	.	.	.	r	.	.	.	.	.	.	.	33
subBo- real	H	6	<i>Geum coccineum</i> Sibth. & Sm.	.	r	.	r	.	2	+	.	1	1	r	r	r	.	.	50
Eur	H	6	<i>Luzula luzuboides</i> (Lam.) Dandy & Willmott	.	2	.	.	.	.	.	.	r	.	.	.	.	r	.	17
Eur	H	6	<i>Stellaria nemorum</i> L.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	17
Alp-Carp- Bal	H	6	<i>Rumex alpinus</i> L.	.	.	r	.	.	.	.	.	.	.	.	1	.	.	.	17
Eur-Med	G-H	6	<i>Doronicum austriacum</i> Jacq.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	17
Bal	H	6	<i>Cirsium appendiculatum</i> Griseb.	r	r	.	.	.	.	.	r	.	.	.	.	r	.	.	33
Diagnostic species of cl. <i>Scheuchzerio palus- tris-Caricetea fuscae</i>																			
Boreal	H	6	<i>Eriophorum latifolium</i> Hoppe	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	0
subBo- real	H	6	<i>Parnassia palustris</i> L.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	0
Alp-Carp	G-H	6	<i>Carex nigra</i> (L.) Reichard	.	.	.	.	r	1	r	2	4	2	1	3	2	.	r	33

subBo- real	Ph	4	<i>Salix caprea</i> L.	.	+	.	2	3	.	.	.	.	r	.	.	.	1	.	.	.	50	18
Eur-As	H	6	<i>Vicia cracca</i> L.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	0	9
Eur-As	H	6	<i>Vicia sepium</i> L.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	17	0

Relevé information (Locality/Latitude/Longitude): Relevé 1: Vitosha Mt., Bistrishko Branishte Reserve/42.55164/23.30707; Relevé 2: Vitosha Mt., along way to Aleko peak/42.5917/23.28907; Relevé 3: Vitosha Mt., above Aleko hut along Bistritsa river/42.57994/23.29104; Relevé 4: Vitosha Mt., above NSA hut/42.54885/23.31401; Relevé 5: Vitosha Mt., near Samara shelter/ 42.56571/23.24993; Relevé 6: Vitosha Mt., near Konyarnika/42.57994/23.24881; Relevé 7: Vitosha Mt., Bistrishko Branishte Reserve, under Goli peak/42.59141/23.28729; Relevé 8: Vitosha Mt., Torfeno Branishte Reserve, along path to Kamen dol peak/42.57994/23.29104; Relevé 9: Vitosha Mt., Bistrishko Branishte Reserve, along way to Aleko peak/42.58434/23.28049; Relevé 10: Vitosha Mt., Torfeno Branishte Reserve/42.587/23.27004; Relevé 11: Vitosha Mt., between Ushite shelter and Golemi vrah peak/ 42.60314/23.2823; Relevé 12: Vitosha Mt., Torfeno Branishte Reserve/ 42.58249/23.27015; Relevé 13: Vitosha Mt., near Konyarnika/ 42.56935/ 23.25271; Relevé 14: Vitosha Mt., W of Kamen del peak/42.60939/23.27023; Relevé 15: Vitosha Mt., above Boeritsa hut/ 42.60072/23.26163; Relevé 16: Vitosha Mt., near Konyarnika/42.57726/23.25049; Relevé 17: Vitosha Mt., Torfeno Branishte Reserve/ 42.58742/23.27493. Used abbreviations: H – hemicryptophytes, Ph – phanerophytes, G – geophytes, G-H – geophytes – hemicryptophytes, Ch – chamaephytes; soil depth: 1 – shallow soils, 2 – moderately deep soils.

Chemical weed control in lavender field

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Thematic field: Agriculture in mountainous and semi-mountainous regions

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Abstract

An open-field study was carried out in 2023–2024 at the Institute for Roses and Aromatic Plants – Kazanlak, aiming at evaluation of herbicide weed control in lavender plantation (*Lavandula angustifolia* Mill.) cultivar 'Yubileina'. Herbicide efficacy and selectivity of BeFlex (500 g·L⁻¹ beflubutamid) and Battle Delta (400 g·L⁻¹ flufenacetate + 200 g·L⁻¹ diflufenican) were determined, applied on soil in two concentrations before beginning of lavender vegetation. The effect of the herbicides on weed infestation and on lavender growth and development was followed. Results analyses show that BattleDelta successfully controls annual dicotyledonous and monocotyledonous weeds (*Chenopodium album* L., *Polygonum aviculare* L., *Veronica agrestis* L., *Sonchus oleraceus* L., *Alopecurus myosuroides* Huds., *Poa annua* L.). The soil activity of the herbicide persists up to the 60th day after treatment. That makes it possible to eliminate the competitive impact of weeds on lavender growth after beginning of growing. BeFlex exerts effective action on annual dicotyledonous weeds reducing their number by 14–17 % compared to the control. No phytotoxicity was observed after herbicide application. On the contrary, lavender fresh mass and oil yields were higher in treated area than from the untreated control.

Key words: beflubutamid, efficacy, flufenacetate, herbicides, *Lavandula angustifolia*, selectivity.

Introduction

Lavender (*Lavandula angustifolia* Mill.) is an essential oil crop that is vastly cultivated in Bulgaria mainly to obtain high-quality essential oil. An important cultivation

practise is weed control since weeds are competitors of crops for water, light and nutrients (Baeva 2017). With a lack of light, the number of flowering stems of lavender decreases, hence fresh flowers yield diminishes, as well. In addition, it

was found that the content of linalyl acetate, a significant component of lavender oil, also decreases (Atanasov et al. 2008).

One of the main problems in lavender cultivation in our country is the limited number of herbicides authorised for use. For this reason, research into new active substances with a broad spectrum of activity and reliable ecological characteristics is necessary in order to optimise integrated weed control in lavender plantations.

Several herbicides that successfully control weeds in lavender and sage plantations were tested in field by Vouzounis et al. (2003) from the Agricultural Research Institute, Nicosia, Cyprus. The trial included application of following herbicides in two concentrations: aclonifen – 2.75 and 3.00 kg·ha⁻¹, chloridazon – 2.00 and 3.00 kg·ha⁻¹ and 0.80 kg·ha⁻¹, oxadiazon – 0.75 and 1.00 kg·ha⁻¹, oxyfluorfen – 0.50 and 0.75 kg·ha⁻¹. High efficiency against weeds was recorded for oxyfluorfen and oxadiazon. Oxyfluorfen was distinguished by better herbicidal action, oxadiazon did not control *Stellaria media* L. Treatments with those herbicides led to an increase in fresh inflorescences yield. Aclonifen showed a toxic effect on lavender expressed in leaves chlorosis, which was overcome at a later stage and did not have a negative effect on yield formation. In other studies, (Angelova and Baeva 2018) was found that the application of flumioxazin (Pledge 50 VP) at a dose of 400 g·ha⁻¹ successfully controlled a large number of annual cereal and broadleaf weeds and does not have a negative effect on the growth and productivity of flowering lavender. In addition, the herbicide oxyfluorfen (Fen 24 EC) at a dose of 1.2 L·ha⁻¹ exhibits 92 % efficacy against weed manifestation in a lavender field (Angelova and Dobрева 2009).

Studies on cereals provide additional

insight into herbicide efficacy and selectivity, which may be relevant for lavender cultivation. Kleinhans (2004) and Rohde et al. (2010) applied 500 g·L⁻¹ beflubutamid (Beflex) in autumn to winter cereals. They found that applied at a dose of 0.5 L·ha⁻¹ in the early stages after crop emergence, it exhibits effective control of broadleaf weeds and is characterised by very good crop safety in all types of cereals. Petrova et al. (2022) found that sole application of beflubutamid was not effective against *Amaranthus caudatus* L., but the combination of Beflex – 0.5 L·ha⁻¹ + Dual Gold – 1.2 L·ha⁻¹ successfully controlled this weed. Gitsopoulos et al. (2024) tested flufenacetate plus diflufenican for weed control in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), applied early after emergence on the crop (1st–3rd leaf growth phase). To evaluate the selectivity of these new herbicides, pot experiments were conducted by applying flufenacetate with diflufenican at a rate of 240 + 120 g a.i.·ha⁻¹. The results also showed reduced selectivity of the herbicides when applied in the BBCH-11 phenophase for both cereals, with flufenacetate + diflufenican being poorly selective but effective chemical options for controlling herbicide-resistant *Lolium rigidum* Gaudin. To provide crop safety and optimise efficacy, application at BBCH-12 phenophase was recommended.

According to Dücker et al. (2019), flufenacetate has become a key herbicide for the control of multi-resistant *Alopecurus myosuroides* Huds. However, reduced efficacy of flufenacetate has already been observed in some of these populations. After a study, they found that the use of flufenacetate in mixtures with diflufenican significantly improved efficacy in less sensitive populations of *A. myosuroides*. This is particularly important as

flufenacetate is one of the few herbicides still effective for controlling multi-resistant genotypes of *A. myosuroides* in Europe, while alternative pre-emergence herbicides are less effective. Soil herbicides have become the mainstay for controlling *A. myosuroides* populations (Menne et al. 2012). Application of flufenacetate with diflufenican and especially flufenacetate alone showed large variations in their effectiveness in field trials in northern Germany. The variation initially depends on soil moisture conditions, temperature and plant growth stage at the time of application. Other factors have an additional influence, such as herbicide dose, treatment time, crop competition, etc. (Hess et al. 2016).

The aim of this study was to evaluate the efficacy and selectivity of BeFlex (500 g·L⁻¹ beflubutamid) and Battle Delta (400 g·L⁻¹ flufenacetate + 200 g·L⁻¹ diflufenican) when applied to soil and their influence on yield of lavender inflorescences and essential oil.

Material and Methods

Plant material and growth analyses

An open-field trial with lavender (*Lavanda augustifolia* Mill., cv. 'Yubileina') was set up at experimental field of the Institute for Roses and Aromatic Plants in Kazanlak, Bulgaria (42°38'07.4" N 25°23'22.9" E), in 2023–2024. The experimental plot contained four-year-old lavender plants and was organised using a block method in four replications with a plot size of 20 m² in the following variants: V1 – control without herbicide and hoeing, V2 – Beflex – 0.5 L·ha⁻¹, V3 – BeFlex – 1 L·ha⁻¹, V4 – Battle Delta – 0.6 L·ha⁻¹, V5 – Battle Delta – 1.2 L·ha⁻¹.

Beflex is a broad-spectrum systemic herbicide for the control of broadleaf weeds in cereal crops. BattleDelta is a selective herbicide for the control of annual broadleaf and annual cereal weeds in wheat, barley and rye. They are applied after sowing before crop emergence or in the early stages of their development.

The herbicides were applied before lavender growing and before the emergence of weeds in the first decade of March. Weed counts were performed using the quantitative method, recording the number of individuals per 1 m². The efficacy of herbicides was assessed on the 30th and 60th day after application. The percentage of destroyed weeds was calculated relative to the untreated control. The selectivity of the herbicides was determined using the EWRS scale, ranging from 1 (no crop damage) to 9 (complete crop destruction).

The herbicide treatment influence on lavender growth and development was evaluated via recording the following indexes: tuft height (cm), tuft diameter (cm), length of inflorescences (cm), number of inflorescences per plant, yield of fresh flowers (kg·ha⁻¹) and yield of essential oil (kg·ha⁻¹). Data were averaged for the 2023–2024 period. The results were statistically analysed, and the differences between the individual variants were established by one-way analysis of variance (Zapryanov and Marinkov 1978).

Essential oil distillation and GC analyses

Lavender inflorescences were air-dried at room temperature. Essential oil was extracted by steam distillation using 10 L distillation units.

The chemical composition of the essential oils obtained from the studied vari-

ants was determined by gas chromatography. Gas chromatographic analysis was performed with an Agilent Technologies gas chromatograph (model 7820A, USA) equipped with a flame ionisation detector and a non-polar EconoCap EC-5+ column (30 m × 0.32 mm, 0.25 µm film thickness). The chromatographic conditions were as follows: oven temperature increased from 40 °C to 240 °C at a rate of 10 °C·min⁻¹. The injector and detector temperatures were set at 200 °C and 300 °C, respectively. Helium, used as the carrier gas, was adjusted to a linear velocity of 32 cm·s⁻¹. Samples were injected using split sampling technique at a ratio of 1:50. Quantitative data were obtained from electronic integration of peak areas without the use of correction factors.

Results and Discussion

Trial period 2023–2024 covers fourth and fifth year after lavender plantation was es-

tablished. Climate analyses were based on data obtained from the synoptic station Kazanlak of the National Institute of Meteorology and Hydrology (NIMH), Bulgaria, for 2023 and 2024. The yearly data were compared to climatic norm for the period 1987–2017. Meteorological factors that mostly influence the growth and development of lavender are average daily air temperatures and total precipitation, as well as their distribution during the growing season. The year 2024 was characterised as typical in terms of optimal temperatures during this period (Fig. 1). During the flowering and harvest period of lavender (June–July), precipitation and high atmospheric humidity lead to a decrease in flower essential oil content (Nedkov et al. 2005). In 2024, a higher amount of precipitation for this period was recorded, but the main quantities in July fell after the harvest. For 2023, increased precipitation during the last ten days of June, together with lower average daily temperatures, adversely affected both the content and

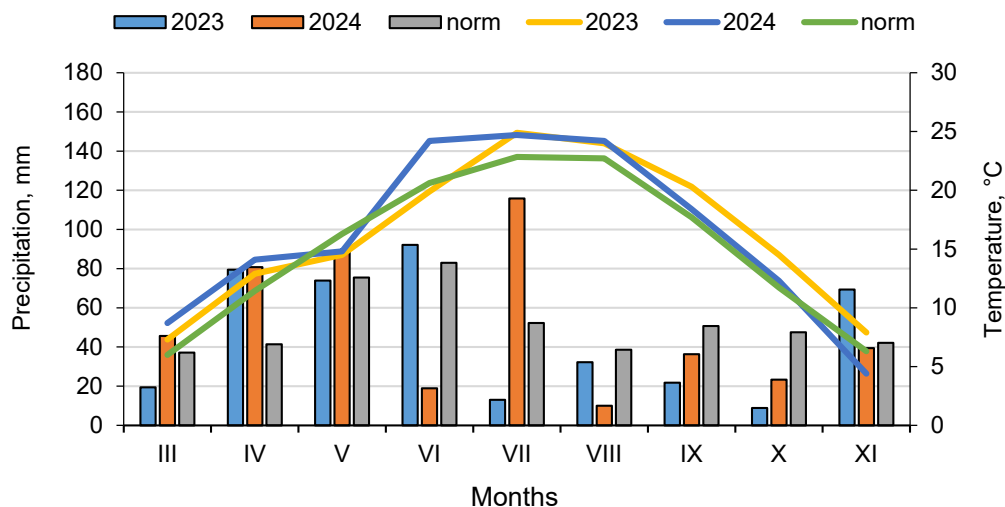


Fig. 1. Meteorological data (average daily air temperature and total precipitation sum) during lavender vegetation in 2023–2024.

Source: Synoptic Station Kazanlak, NIMH, Bulgaria.

quality of the essential oil.

The following annual species prevailed in the weed association of the experimental lavender plot: *Alopecurus myosuroides*, *Poa annua* L., *Polygonum aviculare* L., *Fumaria officinalis* L., *Veronica agrestis* L., *Chenopodium album* L., *Sonchus oleraceus* L., *Capsella bursa-pastoris* L., *Lamium purpureum* L., *Thlaspi arvense* L. The effect of herbicide applications at different dosage on weed diversity is shown in Table 1. Double counting of weeds shows a very good effect of Battle Delta application against the annual

grass weeds *Alopecurus myosuroides* and *Poa annua* with higher dose (V5) reaching 100 % efficacy by the 30th day after treatment. This confirms the results of a study by Dücker et al. (2019) that the use of flufenacet in mixtures with diflufenican significantly improved efficacy in less susceptible populations of *A. myosuroides*. The efficacy of the herbicide is clearly expressed also against the annual dicotyledonous weed species *Polygonum aviculare*, *Fumaria officinalis*, *Veronica agrestis*, *Capsella bursa-pastoris*, *Lamium purpureum*, etc. On the 30th day after

Table 1. Dynamics of weed emergence by species (number per m²) in lavender plantation.

Weed species	30 days					60 days				
	V1	V2	V3	V4	V5	V1	V2	V3	V4	V5
2023										
Annual monocotyledonous weeds										
<i>Alopecurus myosuroides</i> Huds.	7	3	2	2	0	9	2	2	3	1
<i>Poa annua</i> L.	5	2	1	1	0	6	2	1	1	1
Annual dicotyledonous weeds										
<i>Polygonum aviculare</i> L.	10	5	2	1	0	12	3	3	2	1
<i>Fumaria officinalis</i> L.	6	1	0	0	0	7	2	2	2	1
<i>Veronica agrestis</i> L.	5	2	1	0	0	3	2	1	1	0
<i>Chenopodium album</i> L.	8	3	1	2	1	10	5	2	3	1
<i>Sonchus oleraceus</i> L.	6	2	1	1	1	7	3	2	2	2
<i>Capsella bursa-pastoris</i> L.	4	1	0	1	0	6	3	1	3	2
<i>Lamium purpureum</i> L.	5	2	1	2	0	7	3	1	3	1
<i>Thlaspi arvense</i> L.	6	3	2	2	1	8	5	4	4	3
2024										
Annual monocotyledonous weeds										
<i>Alopecurus myosuroides</i>	5	4	1	2	1	7	3	3	3	2
<i>Poa annua</i>	6	1	1	1	0	7	3	1	2	2
Annual dicotyledonous weeds										
<i>Polygonum aviculare</i>	9	3	2	1	1	11	5	3	3	1
<i>Fumaria officinalis</i>	8	2	1	0	1	12	3	4	5	2
<i>Veronica agrestis</i>	4	1	1	0	0	2	2	2	2	0
<i>Chenopodium album</i>	5	2	2	1	1	8	3	4	3	2
<i>Sonchus oleraceus</i>	6	3	0	1	0	9	5	2	2	3
<i>Capsella bursa-pastoris</i>	6	2	0	2	0	7	5	3	3	2
<i>Lamium purpureum</i>	6	3	1	1	0	8	4	3	3	2
<i>Thlaspi arvense</i>	7	2	1	2	1	10	4	4	5	3

treatment, for V5 were recorded 3 pcs·m⁻² and 9 pcs·m⁻² for V4, while in the control variant they reached 50 pcs. The exposition of herbicidal protection of Battle Delta against annual weeds was maintained until the 60th day after treatment, when applied at dose of 1.2 L·ha⁻¹.

BeFlex (V3), applied at a dose of 1.0 L·ha⁻¹, showed excellent biological efficacy against the sensitive annual weeds *Fumaria officinalis* and *Capsella bursa-pastoris* up to the 30th day after treatment. BeFlex at the lower dose of 0.5 L·ha⁻¹ (V4) had a weaker effect, which confirms the data from previous studies (Kleinhans 2004).

The second counting on weed association numbers showed a weakening in herbicidal protection of BeFlex. An increase in the annual weeds *Thlaspi arvense* and *Polygonum aviculare* was observed. A prolonged herbicidal effect was established against *Lamium purpureum*.

The emergence dynamics of weed species composition and the herbicidal efficacy of the applied herbicides were similar in the second year, 2024. The double counting of weeds in 2024 confirmed

the results obtained in 2023 for the good efficacy of Battle Delta against annual weeds. The duration of the soil action of the herbicide is maintained until the 60th day after treatment, with the number of weeds reaching 9 pcs·m⁻².

Lavender growth and development were followed for two years in order to determine the influence of herbicide treatment (Table 2). Tuft height is an indicator that gives an idea of the rate of plant development. The values of this morphological indicator of the herbicide-treated plants ranged from 58 to 60 cm. The highest height was found at V4.

The yield of fresh mass is directly dependent on tuft diameter and inflorescences count. High values of these indicators are a prerequisite for high yields. Regarding tuft diameter, statistical analysis showed that all herbicide-treated variants outperformed the untreated control with the differences being statistically significant. The highest values are distinguished by the variants V2, V4 and V5 (over 85 cm). The plants from the treated variants form a greater number and longer inflorescences compared to the untreated

Table 2. Effect of herbicide treatment on lavender growth and development in 2023–2024.

Variant	Plant height, cm	Significance	Tuft diameter, cm	Significance	Inflorescence length, cm	Significance	Number of inflorescences	Significance
V1	57.3		72.7		6.4		127	
V2	58.0	n.s.	86.7	***	7.2	*	159	**
V3	59.4	n.s.	74.4	**	7.7	**	152	*
V4	60.4	*	88.7	***	7.3	*	182	***
V5	58.1	n.s.	85.1	***	7.6	**	166	**

Significance levels (LSD test):

Plant height: GD₅% = 2.36; GD₁% = 3.83; GD_{0.1}% = 3.83.

Tuft diameter: GD₅% = 1.14; GD₁% = 1.66; GD_{0.1}% = 2.49.

Inflorescence length: GD₅% = 0.79; GD₁% = 1.15; GD_{0.1}% = 1.73.

No. of inflorescences: GD₅% = 20.13; GD₁% = 29.28; GD_{0.1}% = 44.00.

Abbreviations: n.s. – not significant; * – significant at $p < 0.05$; ** – significant at $p < 0.01$; *** – significant at $p < 0.001$.

ed ones. The variant with Battle Delta – $0.6 \text{ L} \cdot \text{ha}^{-1}$ (V4) surpasses all others and the control in terms of the number of inflorescences.

External symptoms of phytotoxicity and visible disturbances in the development of treated lavender variants were not observed.

The differences in the productive qualities of the individual variants in terms of fresh flower yield are shown by one-way analysis of variance (Table 3). In treated variants, the yields of fresh inflorescences ranged from 5013 to $5283 \text{ kg} \cdot \text{ha}^{-1}$ in 2023 and from 7323 to $7922 \text{ kg} \cdot \text{ha}^{-1}$ in 2024. Throughout the study period, all treated variants outperformed the control, with statistically significant differences.

The amount of essential oil, obtained per hectare, varied among variants and depended on both flower yield and oil content in the flowers. It has been established that the essential oil yield of treated variants exceeded that of the controls by 2–11 % in 2023, and by 33 to 47 % in 2024.

Table 4 presents the results of gas chromatographic analysis of the essential oils composition obtained from lavender fresh inflorescences. No significant differences in the values of the individual components have been established, both between the individual variants and between the treated ones and the control. All essential oils, with very few exceptions, comply with the accepted standard for Bulgarian lavender oil (ISO 3515:2002).

Conclusions

Battle Delta at $1.2 \text{ L} \cdot \text{ha}^{-1}$ dose provided effective control against annual weeds in open field treatment. The herbicide's persistence in soil was maintained until the 60th day after treatment.

BeFlex, applied at a dose of $1.0 \text{ L} \cdot \text{ha}^{-1}$, demonstrated good biological efficacy against sensitive annual weeds until the 30th day after treatment.

Herbicides application led to an increase in height, diameter, yield of fresh

Table 3. Effect of herbicide treatment on lavender fresh flower and essential oil yields in 2023–2024.

Variants	Flower yield, kg				Essential oil yield, kg			
	2023	Warranted	2024	Warranted	2023	Warranted	2024	Warranted
V1	4803		6483		77.297		90.943	
V2	5037	*	7323	***	78.677	n.s.	121.262	***
V3	5123	*	7922	***	85.077	**	125.180	***
V4	5013	n.s.	7793	***	84.320	**	133.923	***
V5	5283	**	7740	***	85.980	***	127.361	***

Significance levels (LSD test):

Flower yield:

2023 – $\text{GD}_5\% = 226$; $\text{GD}_1\% = 330$; $\text{GD}_{0.1}\% = 494$.

2024 – $\text{GD}_5\% = 231$; $\text{GD}_1\% = 336$; $\text{GD}_{0.1}\% = 505$.

Essential oil yield:

2023 – $\text{GD}_5\% = 3.947$; $\text{GD}_1\% = 5.743$; $\text{GD}_{0.1}\% = 8.630$.

2024 – $\text{GD}_5\% = 3.521$; $\text{GD}_1\% = 5.123$; $\text{GD}_{0.1}\% = 7.698$.

Abbreviations: n.s. – not significant; * – significant at $p < 0.05$; ** – significant at $p < 0.01$; *** – significant at $p < 0.001$.

Table 4. Influence of herbicide treatment on composition of lavender essential oil.

Variants	3-octanone	Cis- β -ocimene	Trans- β -ocimene	Linalool	Camphor	Lavandulool	Terpinen-4-ol	Linalyl acetate	Lavandulyl acetate
V1	1.49	5.22	2.10	27.12	0.59	1.19	6.42	35.27	4.59
V2	1.47	5.30	3.21	24.29	0.39	0.94	5.75	38.01	4.02
V3	1.38	5.29	3.15	25.21	0.37	0.99	6.63	37.40	3.86
V4	1.21	4.73	3.10	25.88	0.44	1.11	6.83	35.11	3.74
V5	1.50	4.13	2.72	25.84	0.37	0.83	6.68	37.17	4.90
ISO 3515	0.2– 1.6 %	3.0– 9.0 %	2.0– 5.0 %	22.0– 34.0 %	≤0.6 %	≥0.3 %	2.0– 5.0 %	30.0– 42.0 %	2.0– 5.0 %

Note: All values represent percentages of the total essential oil composition, except for the ISO 3515 row, which indicates the standard limits for Bulgarian lavender oil.

inflorescences and essential oil of lavender plants per unit area. The applied herbicides did not affect the quality of lavender essential oil.

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Contemporary landscape design for the rehabilitation of the University of Forestry's Petrohan Arboretum

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Thematic field: Landscape architecture

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Abstract

Botanical gardens and arboreta play an important role in plant science research and biodiversity conservation. In recent decades, the social role and cultural functions of botanical gardens, as urban green infrastructure elements and tourist attractions, have influenced their design. The University of Forestry's Petrohan Arboretum serves primarily as an educational resource. In addition, it plays a key role in scientific research on tree species' responses to climate change and their adaptation to the local environment. The beautiful nature makes the campus attractive as a centre for workshops, seminars, and green schools. It has great potential for further development, particularly as an attractive destination for educational tourism. The aim of this research is to develop a contemporary landscape design concept for the rehabilitation of the University of Forestry's Petrohan dendrological park and its transformation into both a functional hub for education and research, and an inspiring green space that enhances the landscape's aesthetic value while promoting biodiversity. The preliminary studies involved data collection and analysis of the current condition of vegetative and non-vegetative elements. The key design principles for the rehabilitation of the Arboretum were: (1) enhancing biodiversity; (2) providing engaging educational opportunities; (3) developing a sustainable design. These principles emphasise the importance of creating enriching, multifunctional nature-based open spaces that not only support biodiversity and scientific research, but also serve as educational and recreational resources for students and the community. A master plan following the new concept of functions and structural planning has been designed based on the site analysis.

Key words: biodiversity, botanical garden, dendrology, landscape architecture, tree selection.

Introduction

Botanical gardens and arboreta play an important role in plant science research, biodiversity conservation, and botanical and horticultural education (Faraji and Karimi 2022, Hohn 2022, Sjöman et al. 2025).

Additionally, they significantly contribute to the great diversity of public green spaces for recreation, to the urban landscape aesthetics, and to the economy as tourist attractions (van den Toorn 2017, Yücel 2020, Faraji and Karimi 2022, Elma et al. 2024). They have a social and cultural function, playing a vital role in the development of public awareness on a wide range of topics related to environmental protection (Hohn 2022). For instance, the implementation of bioblitzes in arboreta can facilitate rapid data collection on local biodiversity, enhancing conservation efforts and fostering collaboration among diverse stakeholders (Parker et al. 2018).

All the oldest European botanical gardens and arboreta were established by the major universities of the cities in which they were located (Neves 2024).

The scientific approach to plant species arrangement and display in botanical gardens and arboreta follows systematic classification, phytogeographical regions, ecological groupings, uses, etc. (Hohn 2022). In recent decades, the role and functions of botanical gardens as part of the urban green infrastructure have changed. Currently, these design principles for arrangement by a certain classification are not followed so strictly because they do not contribute to the creation of a special atmosphere, aesthetic value, and identity of these public green spaces, and interfere with their use (van den Toorn 2010, 2017; Eminağaoğlu and Eminağaoğlu 2024). Contemporary design

approach at the structural level focuses on continuity in the long run and developments and interventions in the short term (van den Toorn 2017).

Arboreta serve as living laboratories for students, allowing them to apply ecological design principles in real-world settings and thereby enhancing their learning experience. They could be designed to be more accessible and engaging for university communities and utilised to expand informal botanical learning (Melhem et al. 2023).

The University of Forestry's Petrohan Arboretum serves primarily as an educational resource for the disciplines of dendrology and ornamental dendrology. In addition, it plays a key role in scientific research on tree species' responses to climate change and their adaptation to the local environment. It is open to the public. The beautiful nature makes the campus attractive as a centre for workshops, seminars, and green schools.

The aim of this research is to develop a contemporary landscape design concept for the rehabilitation of the University of Forestry's Arboretum and its transformation into both a functional hub for education and research and an inspiring green space that enhances the landscape's aesthetic value while promoting biodiversity.

Materials and Methods

The site plan of the University of Forestry's Petrohan Arboretum was developed by Galev et al. (2012) using remote sensing, including very high-resolution satellite images from Google Earth, and GIS technologies.

The preliminary studies, conducted before developing the design concept, were related to collecting data and performing

analysis about the current condition of vegetative and non-vegetative elements.

Soil samples were collected from the main root-containing layer (0–55 cm).

Mapping, inventory, and on-site identification of the existing woody specimens in the dendrological collection were carried out, along with a phytosanitary status assessment, following the methodology of Pencheva (2015) (Table 1).

A systematic analysis of gymnosperm and angiosperm species was made. In order to establish the principle applied in the arboretum's masterplan, three graphical analyses of the dendrological collection were performed: a systematic/taxonomic analysis by family, an analysis by range, and an analysis of the origin (native, non-native) of each specimen.

The need to expand and enrich the plant collection according to the academic curricula of the Forestry, Landscape Architecture, Ecology, Agronomy, and Plant Protection programmes, and to align it with local climate and soil conditions, was analysed.

An analysis of the pathway network and accessibility to different sectors was made.

As a result, a master plan following the new concept of functions and structural planning for the arboretum has been de-

signed based on the site analysis.

Results and Discussion

History

The Arboretum of the University of Forestry was established in 1976. The landscape architectural site plan was developed by landscape architect Anastasia Shevkenova.

The limiting factors for the development of the species in the collection are soil conditions, temperature, and drought. Additionally, heavy snowfalls, such as those in the winter of 2015, fell a large number of mature coniferous trees.

Physico-geographical characteristics, location and area

The arboretum is situated in northwestern Bulgaria, in the western part of the Balkan Mountains, within the 'Petrohan' Training and Experimental Forest Range (43°11'19.5" N and 23°09'14.3" E).

After measurements were made with the HI-TARGET GNSS V200 RTK SYSTEM, it was found that the average slope of the arboretum area is nearly 12 %, and the mean altitude is 540 m a.s.l. Its area is

Table 1. Scale for assessing tree damage (Pencheva 2015)

Tree condition	Value	Main characteristics/indicator
Drying, dying or dead tree	4	Specimen with low vitality, low ornamental value, damage, and crown defoliation over 96 %.
Tree in poor condition	3	Individual with insignificant ornamental value, low vitality and crown damage and defoliation between 67 % and 95 %.
Tree in unsatisfactory condition	2	Viable/vigorous specimen with good ornamental value and crown damage and defoliation from 26 % to 66 %.
Tree in satisfactory condition	1	Individual with high degree of vitality and aesthetic value, crown damage and defoliation from 11 % to 25 %.
Healthy tree	0	High degree of vitality and excellent aesthetic value, with crown damage and defoliation up to 10 %.

3.1 ha, including the representative green areas around the main buildings. The desirable features of arboreta that provide landscape diversity and different perspectives – sloping terrain, river, pond (Ateş and Sabaz 2013) – are all present within the area of the dendrological park of the ‘Petrohan’ Training and Experimental Forest Range (Fig. 1).

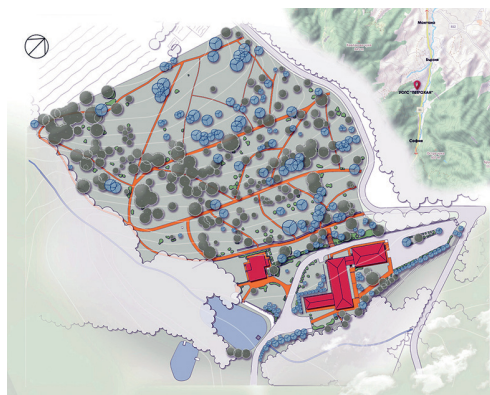
Climatic characteristics

According to the climate classification of Bulgaria, the study area is part of the European-continental climate region, the Temperate-continental climate subregion and the premontane climate region (Sabev and Stanev 1963).

The mean annual temperature in the study area is 10.4 °C (Berkovitsa station). The coldest month is January, with average temperature of -2.2 °C, and the warmest is July, with average value of 21.2 °C for Berkovitsa. The period with stable ambient temperatures above 5 °C lasts about 220–240 days, while temperatures above 10 °C occur for about 170–190 days (BCRB 1983). The mean annual precipitation is 957 mm (station

Barzia). The maximum precipitation is in the spring-summer season, and the minimum – in winter (CRB 1990). The mean annual relative humidity is 76 % for Berkovitsa. The studied area exhibits the typical features of the Eastern European continental climate (for the respective latitude and altitude) – relatively cold winters and relatively warm summers.

According to the climatic division of Bulgaria based on De Martonne aridity index and the Thornthwaite moisture index (Mitkov and Topliyski 2023), the surroundings of the ‘Petrohan’ Training and Experimental Forest Range have the following characteristics: under the De Martonne climate classification scheme, the territory is characterised by an Extremely Humid (a) climate type, bordering on the Extremely Humid (b) climate type due to higher annual precipitation (957 mm, Barzia station). The Thornthwaite index places the study territory in the B1 – Humid climate type. On an annual basis, both indices indicate moisture conditions ranging from very good to excessive. The monthly values of the De Martonne index, based on temperature data from the Berkovitsa station and precipitation data from the Bar-



A) Site plan – current situation.



B) Panoramic view.

Fig. 1. Dendrological park of the ‘Petrohan’ Training and Experimental Forest Range.

zia station (for July, August, and September), are 33.1, 24.8, and 30.5, respectively. Thus, the index during these months ranges from Semihumid (only in August) to Humid (in July and September). The climatic diagram of Barzia, following Walter (1963), confirms the absence of a moisture deficit during the growing season (Fig. 2).

The De Martonne and Thornthwaite indices are of considerable theoretical and practical significance (Mitkov and Topliyski 2023).

Based on the climatic characteristics, it can be concluded that the environmental conditions of the study area allow the cultivation of a wide diversity of woody species, which contributes to an increase in local biodiversity.

Soil characteristics

According to the Soil Division of Bulgaria and the conducted field survey, the natural soils of the study area belong to the

Grey Forest Soils (Luvisols) class in the FAO/WRB (World Reference Base for Soil Resources) system. The soils have formed over gneiss parent material. They are strongly acidic (with pH values ranging from 4.8 in the L horizon to 3.7 in the BC horizon), heavily eroded in the A horizon, and poor in nutrients, especially in the deeper horizons. The humus content is moderate to low in the surface layers (4.03 % in the A horizon and decreasing with depth to 1.14 % in the BC horizon). The soils are significantly compacted, especially in the Bt horizons, which may impede drainage and root penetration. Carbonates are absent in all horizons, a typical feature of leached, strongly acidic soils. The C/N ratio ranges between 14 and 20, indicating slow mineralisation and decomposition of organic matter.

These conditions require the implementation of erosion control measures, careful species selection, and the use of soil amendments.

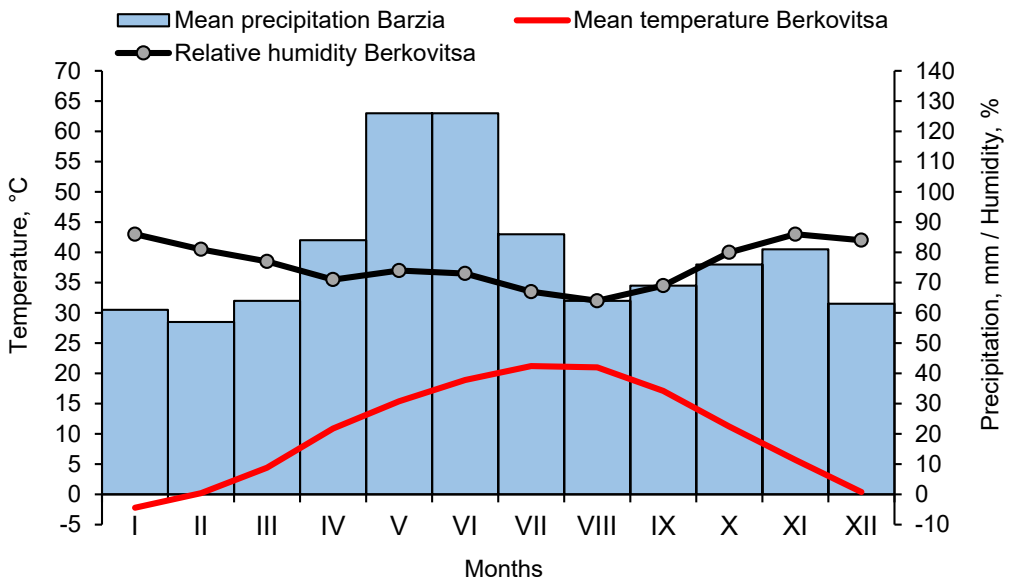


Fig. 2. Climatic diagram of Barzia, following Walter (1963).

Existing woody vegetation

In terms of forest vegetation, the territory of the arboretum falls within the Moesian forest vegetation region, subregion of Northern Bulgaria, and is located at the transition from the hilly pre-Balkan sub-belt of mixed deciduous forests to the low-mountainous belt of common beech, sessile oak, European silver fir, and Scots pine forests (Zahariev et al. 1979).

According to the Forest Management Plan of the 'Petrohan' Training and Experimental Forest Range (Executive Forests Agency 2015), within its territory the main tree species is the common beech (*Fagus sylvatica* L.), which makes up about 80 % of the forest vegetation. In addition, there are common hornbeam (*Carpinus betulus* L.), sessile oak (*Quercus petraea* (Matt.) Liebl.), sweet chestnut (*Castanea sativa* Mill.), Norway maple (*Acer platanoides* L.), sycamore maple (*Acer pseudoplatanus* L.), silver birch (*Betula pendula* Roth), linden, European aspen (*Populus tremula* L.), Turkey oak (*Quercus cerris* L.), pedunculate oak (*Quercus robur* L.), field elm (*Ulmus minor* Mill.), oriental hornbeam (*Carpinus orientalis* Mill.), manna ash (*Fraxinus ornus* L.). Coniferous species include Scots pine (*Pinus sylvestris* L.) and black pine (*Pinus nigra* J.F.Arn.), Norway spruce (*Picea abies* (L.) H. Karst.), European silver fir (*Abies alba* Mill.), and eastern white pine (*Pinus strobus* L.). Shrub vegetation is represented by hawthorn, black elder, common hazel (*Corylus avellana* L.), and dog rose (*Rosa canina* L.).

The detailed inventory of the existing dendrological collection within the boundaries of the arboretum identified 350 individuals of 118 species and intraspecific taxa.

Analysis of exposition principles

The graphical analysis of the arboretum's masterplan (Fig. 3) showed that the arrangement of specimens within the dendrological collection did not follow a systematic (by family belonging) or geographical principle (by range and origin), nor did it adhere to any other criteria. The physical relocation or removal of established species is neither recommended, nor justified.

The participation of family representatives is as follows:

Pinophyta (Gymnospermae)

Among the gymnosperm families, the Cupressaceae was represented by 5 genera, 12 species, and 4 cultivars (*Chamaecyparis lawsoniana* (A.Murray) Parl., *Chamaecyparis lawsoniana* 'Golden Wonder', *Chamaecyparis pisifera* 'Plumosa', *Cupressus arizonica* Greene, *Juniperus virginiana* L., *Juniperus chinensis* L., *Juniperus sabina* L., *Platycladus orientalis* (L.) Franco, *Thuja occidentalis* L., *Thuja occidentalis* 'Columna', *Thuja occidentalis* 'Smaragd', *Thuja plicata* Donn ex D.Don), followed by Pinaceae – 4 genera, 12 species (*Abies cephalonica* Loudon, *Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr., *Cedrus atlantica* (Endl.) Manetti ex Carrière, *Cedrus deodara* (Roxb. ex D.Don) G.Don, *Picea abies* (L.) H. Karst., *Picea pungens* Engelm., *Picea pungens* 'Glauc', *Pinus coulteri* D.Don, *Pinus nigra* J.F.Arnold, *Pinus pinaster* Aiton, *Pinus ponderosa* Douglas ex C.Lawson, *Pinus sylvestris* L., *Pinus strobus* L.); Taxodiaceae – 3 genera, 3 species (*Cryptomeria japonica* (L.f.) D.Don, *Sequoiadendron giganteum* (Lindl.) J.Buchholz, *Metasequoia glyptostroboides* Hu & W.C.Cheng); Ginkgoaceae – 1 (*Ginkgo biloba* L.).

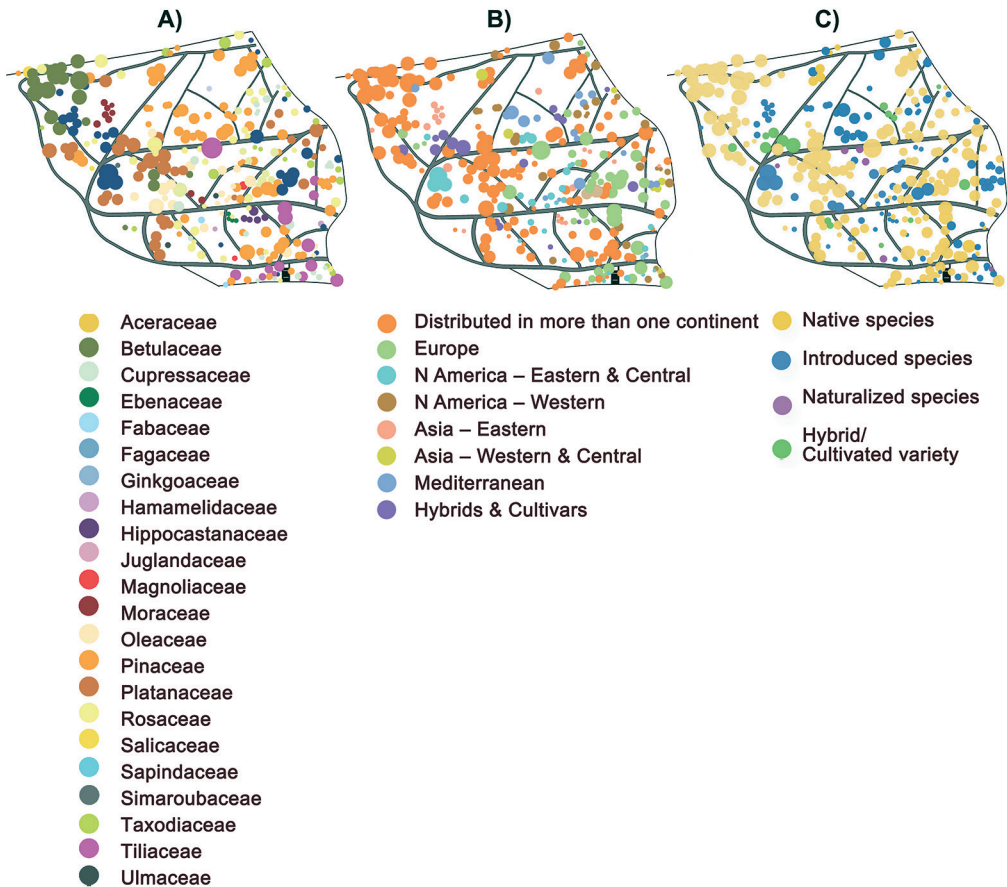


Fig. 3 Graphic analysis of the exposition principles of the specimens within the arboretum: A) systematic – by family; B) geographical – by range; C) geographical – by origin.

Magnoliophyta (Angiospermae)

Amongst the Angiosperm families, the Rosaceae were represented by 9 genera, 17 species, and 1 hybrid (*Cotoneaster dammeri* C.K.Schneid., *Crataegus crus-galli* L., *Malus domestica* (Suckow) Borkh., *Malus silvestris* Mill., *Padus racemosa* (Lam.) Gilib., *Prunus avium* L., *Prunus cerasifera* Ehrh., *Prunus cerasus* L., *Prunus domestica* L., *Prunus serrulata* Lindl., *Pyracantha coccinea* M.Roem., *Pyrus communis* L., *Sorbus aucuparia* L., *Sorbus domes-*

tica L., *Sorbus torminalis* Crantz, *Spiraea* × *billardii* Hérincq, *Spiraea japonica* L. f., *Spiraea* × *vanhouttei* (Briot) Carrière), followed by Fagaceae – 3 genera, 8 species, and 2 cultivated varieties (*Fagus sylvatica* 'Atropunicea', *Castanea sativa* Mill., *Quercus frainetto* Ten., *Quercus hartwissiana* Steven, *Quercus petraea* (Matt.) Liebl., *Quercus protoroburoides* Donc. & Baurov, *Quercus robur* L., *Quercus robur* 'Fastigiata', *Quercus rubra* L.); Oleaceae – 4 genera, 7 species, 1 hybrid, and 1 cultivated variety (*Forsythia* × *intermedia*

Zabel, *Fraxinus americana* L., *Fraxinus angustifolia* Vahl, *Fraxinus excelsior* L., *Fraxinus ornus* L., *Fraxinus pennsylvanica* Marshall, *Ligustrum ovalifolium* 'Aureum', *Syringa vulgaris* L.); Fabaceae – 5 genera and 5 species (*Amorpha fruticosa* L., *Cercis siliquastrum* L., *Gleditsia triacanthos* L., *Laburnum anagyroides* Medik., *Robinia pseudoacacia* L.); Berberidaceae – 1 genera, 3 species, and 1 hybrid (*Berberis thunbergii* DC., *Berberis thunbergii* 'Atropurpurea' DC., *Berberis vulgaris* L., *Berberis* × *ottawensis* C.K.Schneid., *Berberis aquifolium* Pursh); Betulaceae – 4 genera and 4 species (*Alnus glutinosa* Gaertn., *Betula pendula* Roth, *Carpinus betulus* L., *Corylus avellana* L.); Caprifoliaceae – 3 genera and 4 species (*Lonicera fragrantissima* Lindl. et Paxt, *Lonicera nitida* Wils., *Symphoricarpos orbiculatus* Moench, *Viburnum opulus* L.); Sapindaceae – 2 genera, 3 species, and 1 hybrid (*Acer palmatum* Thunb., *Acer pseudoplatanus* L., *Aesculus hippocastanum* L., *Aesculus* × *carnea* Hayne); Platanaceae – 1 genera, 2 species, and 1 hybrid (*Platanus occidentalis*, *Platanus orientalis*, *Platanus* × *acerifolia*); Anacardiaceae – 1 genera, 1 species, and 1 cultivar (*Cotinus coggygia* Scop., *Cotinus coggygia* 'Royal Purple'); Hydrangeaceae – 2 genera and 2 species (*Deutzia crenata* Sieb. et Zucc., *Hydrangea hortensis* Salisb.); Moraceae – 2 genera and 2 species (*Morus alba* L., *Maclura pomifera* (Raf.) C.K.Schneid.); Magnoliaceae – 2 genera and 2 species (*Magnolia kobus* DC., *Liriodendron tulipifera* L.); Salicaceae – 2 genera and 2 species (*Populus tremula* L., *Salix cinerea* L.). The other families were each represented by 1 species and 1 genera: Bignoniaceae – *Catalpa bignonioides* Walter; Cannabaceae – *Celtis australis* L.; Celastraceae – *Euonymus japonicus* Thunb.; Cornaceae – *Cornus sanguinea* L.; Ebenaceae – *Diospyros*

lotus L.; Hamamelidaceae – *Liquidambar styraciflua* L.; Juglandaceae – *Juglans regia* L.; Rhamnaceae – *Frangula alnus* Mill.; Rutaceae – *Poncirus trifoliata* L.; Simaroubaceae – *Ailanthus altissima* (Mill.) Swingle; Tiliaceae – *Tilia tomentosa* Moench; Ulmaceae – *Zelkova sinica* Schneid.

The range of plant species is diverse (Krüssmann 1984, 1985), with the largest group by natural distribution comprising those found across more than one continent (37.4 %), followed by species from Eastern Asia (18.92 %), hybrids and cultivars (14.41 %), and species native to the eastern and central United States (10.81 %).

According to the geographical principle of origin, the following species have been established: native species: 42; introduced species: 55, 10 of which naturalised and 4 of them invasive (Petrova et al. 2012); hybrids and cultivated varieties: 16.

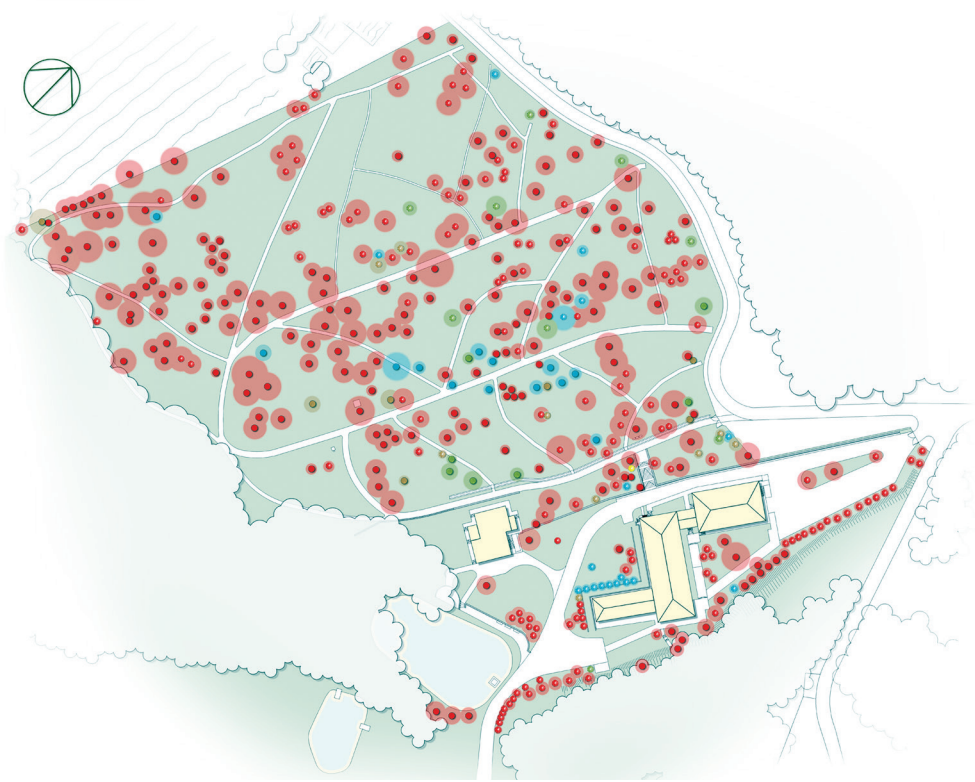
Phytosanitary assessment

The tree health assessment served as a basis for redesigning the existing dendrological collection. Over 350 individual specimens were visually assessed following the methodology of Pencheva (2015) – Table 1. The results revealed that over 80 % of the specimens exhibited normal growth and were in excellent overall condition (Fig. 4).

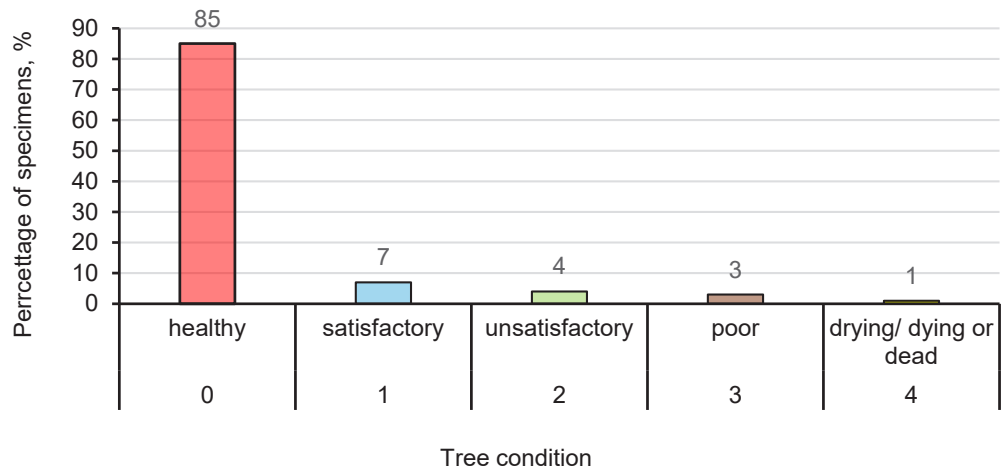
Arboreta can play a significant role in developing plant protection strategies and conducting phytosanitary monitoring (Isikov et al. 2021).

Landscape design for the rehabilitation of the University Arboretum in Petrohan

The rehabilitation plan addresses several key challenges, including the deteriorat-



A) Graphic analysis of trees' phytosanitary status.



B) Average trees condition.
Fig. 4. Phytosanitary status of the trees.

ed pathway network, limited accessibility, and the need to expand and enrich the plant collection.

Key improvements to the alley network focus on creating logical and non-conflicting connections between the various sectors of the arboretum (Fig. 5). One of the main challenges in developing the vertical planning model was the terrain slopes exceeding 12 %. For better service of the territory, a second entrance/exit is planned in the northwestern part of the site, which provides easy access to all thematic zones and recreational areas

in the northern part of the arboretum. The newly designed eastern entrance ensures accessibility for people with disabilities.

The key design principles for the rehabilitation of the University of Forestry's Petrohan Arboretum were as follows:

- 1) enhancing biodiversity;
- 2) providing engaging educational opportunities;
- 3) developing a sustainable design.

Following the first principle, the new dendrological plan incorporates a total of 339 species and intraspecific taxa, including 292 woody ornamentals, carefully se-

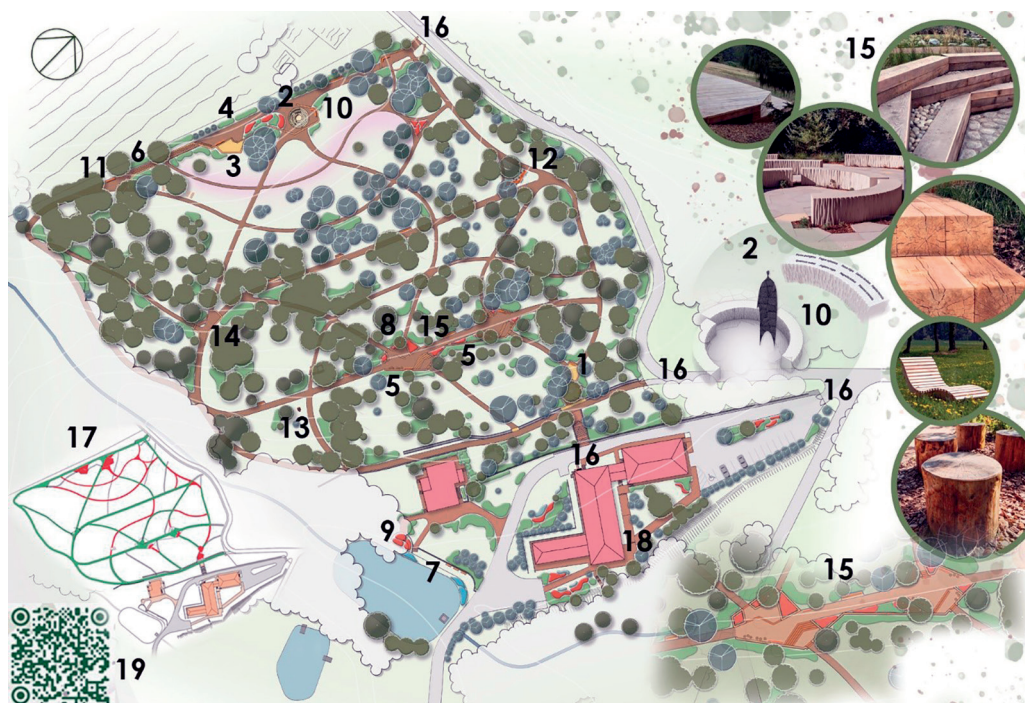


Fig. 5. Landscape design for the rehabilitation of the Petrohan Arboretum, University of Forestry.

Note: 1 – arboretum map; 2 – outdoor classroom; 3 – observation deck; 4 – hedge exhibition; 5 – ground-cover species exhibition; 6 – liana exhibition; 7 – exhibition of hygromesophytic species; 8 – Spirarium; 9 – Rosarium; 10 – thematic zone 'Dendrochronology'; 11 – thematic zone 'Soil Science'; 12 – thematic zone 'Petrography'; 13 – thematic zone 'Meteorology'; 14 – thematic zone 'Ornithology'; 15 – central gathering space, composed of two adjoining squares; 16 – entrance; 17 – comparative plan of existing and proposed alley network; 18 – main campus building; 19 – app with a QR code.

lected to align with local climatic conditions and the academic curricula of the Forestry, Landscape Architecture, Ecology, Agronomy, and Plant Protection programmes. The diverse woody species composition will attract a variety of local wildlife.

Following the second principle, the key innovations include the creation of engaging educational exhibits focused on topics such as soil science, petrography, meteorology, dendrochronology, and ornithology. Additional features include a liana display on a pergola structure, a hedge exhibition, a collection of hygromesophytes near the lake, ground-cover species, a spirarium, a rosarium, and a dedicated gymnosperm exhibit. Highlights of the landscape architectural design include a welcoming decking platform at the arboretum entrance and a central gathering space composed of two adjoining squares, accommodating groups of 30–40 people. In the north-western section, a uniquely designed outdoor classroom will support open-air learning. To enhance learning and engagement, interpretive signage will be supplemented by a mobile app featuring a QR code system, providing detailed information on plant species and their locations throughout the arboretum.

Following the third principle, the primary goal of the landscape design was to preserve the arboretum's existing collection while enhancing accessibility and enriching the visitors' experience through upgraded infrastructure and the introduction of new thematic zones. The new landscape design considered ecological and economic sustainability in terms of construction, management, and maintenance. The construction materials of the designed architectural elements were natural (wood and stone) and locally sourced, harmonising with the surrounding landscape. All newly designed alleys are planned to be

constructed of local permeable solid sandstone pavers, which will ensure durability and aesthetic value. One of the main goals of the design is to create an attractive place for educational tourism.

The design process for arboreta should focus on ecological frameworks that prioritise sustainability and the integration of diverse plant collections (Hurley-Kurtz 2001).

Conclusions

The University of Forestry's Petrohan Arboretum has great potential for further development, including becoming an attractive destination for educational tourism.

The contemporary landscape design principles applied in the rehabilitation of botanical gardens and arboreta focus on biodiversity conservation, educational engagement, and sustainable design. These principles emphasise the importance of creating enriching, multifunctional nature-based open spaces that not only support biodiversity and scientific research, but also serve as educational and recreational resources for students and the community.

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Ground beetle (Coleoptera: Carabidae) communities from various habitats in a region where nature and people collide

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Thematic field: Ecosystem biodiversity, conservation and environmental restoration

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Abstract

'Golak' hill and Karkariya ridge of the Western Rhodope Mts. are subjected to high anthropogenic load from various activities (now and in the past). However, a number of protected species live there, which procured for targeted monitoring. Between March 2023 and June 2024, we surveyed nine sampling plots via 54 pitfall traps and manual collecting of material. Here we present the data obtained about one of the indicative groups of animals, the ground beetles (Coleoptera: Carabidae). A total of 3229 specimens from 92 species were collected. *Calathus distinguendus* Chaudoir, 1846 and *Carabus convexus* Fabricius, 1775 were eudominants; *Carabus montivagus* Palliard, 1825 and *Laemostenus cimmerius* (Fischer von Waldheim, 1823) were dominants. *Carabus convexus* was euconstant, collected in all sampling sites. Beetles belonged to 22 categories from the five faunal types of Bulgarian carabids, as the European-Asiatic (30 species; 33 %) and Mediterranean (21; 23 %) complexes prevailed. We found relatively low number of winged beetles (57 %), which is comparable only with typical montane habitats. The established ratio between the life forms classes (54 % Zoophagous and 46 % Mixophytophagous species) is close to the typical for the meadow steppes from the Forest-steppe zone of Eurasia. In relation to their humidity preferences, mesoxerophilous (38 %) and mesophilous (28 %) carabids predominated. Mathematical processing showed that: 1) there was a clear separation of the forest habitats, based on two indicator species; 2) overgrazed habitats formed another well-defined group; 3) in addition to the habitat type separation, the ecotone edge effect and ecological catastrophic effect were both present, as well.

Key words: bioindication, carabids, ecology, Karkariya, overgrazing, Western Rhodope Mts.

Introduction

'Golak' is a karst hill representing a natural transition zone between Thracian Lowland and the northern foothills of Karkariya Ridge, a part of the Western Rhodope Mountains in Bulgaria. The area is populated, as the hill is in close proximity with the villages of Patalenitsa (to the east) and Vetren Dol and Varvara (to the west). The area is rich in artefacts and architectural structures that are historical evidence of its occupation since ancient times and early beginnings of the exploitation of its natural resources. The ancient Thracian tribes appreciated the unique settlement site, which was subsequently developed into one of the largest fortress towns in the region – Batkunion. In the distant historical past, the local population has skilfully and practically exploited the unique combination of natural resources (hot mineral springs, fertile riverside land, deposits of non-ferrous metal ore, a year-round mild climate) and geostrategic location (natural protection from the possibilities of vandalistic raids and nomadic plunder). The region falls in Pazardzhik Province (Pazardzhik and Stamboliyski Municipalities) and is known with its agriculture and tourism, and currently, intensive cattle-rearing is endangering this karst habitat with overgrazing. In addition to overgrazing, another extremely negative effect of large and unregulated numbers of grazing animals is the trampling, which creates conditions for soil erosion and riparian habitat destruction. Furthermore, the area is open to investment proposals for the extraction of marble, limestone rubble and rare metals. However, it is also a home to a number of protected species requiring priority protection of their habitats (Kodzhabashev et al. 2023), which gave us the reason to conduct a

continued targeted monitoring with the aim of declaring the area protected (Protected Site 'Karkariya') and including it in the NATURA 2000 network.

In the autumn of 2022, we were hired by local initiative of citizens from the village of Vetren Dol ('Karkaria' Association) to map the fauna of the area. Four protected vertebrate species were recorded: Hermann's tortoise (*Testudo hermanni* Gmelin, 1789), Greek tortoise (*T. graeca* Linnaeus, 1758), imperial eagle (*Aquila heliaca* Savigny, 1809) and European souslik (*Spermophilus citellus* (Linnaeus, 1766). Subsequently, in the spring of 2023, the fire salamander (*Salamandra salamandra* (Linnaeus, 1758), European pond turtle (*Emys orbicularis* (Linnaeus, 1758), Montagu's harrier (*Circus pygargus* (Linnaeus, 1758), marbled polecat (*Vormela peregusna* (Gueldenst., 1770) and wolf (*Canis lupus* Linnaeus, 1758) were added to that list (Kodzhabashev et al. 2023). In due time we informed the Ministry of Environment and Water, via the Regional Inspectorate of Environment and Water – Pazardzhik, and the local authorities about our research and about the necessity, at this stage, to suspend all investment procedures for the physical destruction of the hill until the monitoring is completed and a competent decision is made about the admissibility of the enterprises and the conservation significance of the discussed area.

One of the object groups of the zoomonitoring were the ground beetles (Coleoptera: Carabidae). They are one of the globally recognised bioindicator taxa. Many studies suggest that carabids meet broad bioindicator requirements (they are globally widespread and fill several key ecological niches within ecosystems, they are relatively easy to collect, taxonomically and ecologically relatively well studied

and, besides, they occur in high numbers, which allows greater statistical power). Subsequently, they have been used as an object in various bioindication and biodiversity surveys (see Koivula et al. 2002, Koivula 2011, Larochelle and Lariviere 2003, Rainio and Niemelä 2003, Teofilova 2020a). Chowdhury et al. (2023) said that carabids are the most popular insects utilised in environmental biomonitoring, and according to Ludwiczak et al. (2020), they are highly sensitive to any anthropogenically induced transformations. They react quickly to management practices in grasslands and forests, including grazing (Rainio and Niemelä 2003). They were also found a keystone bioindicators of successful land management strategies in restored grasslands and can be used for the assessment of grazing (Waite et al. 2022).

Carabids' distribution in Bulgaria is relatively well studied and certain species, which presence or absence is indicative of a certain anthropogenic impact, can be used as qualitative indicators of the environment. This study aimed at investigating, assessing and presenting the data obtained about this indicative group of invertebrates and their communities inhabiting the region of Golak Hill and the adjacent scrub and forest habitats in Karkariya Ridge of the Western Rhodope Mts., which, although significant in supporting many protected species of animals and plants, are subjected to intensive use by humans.

Materials and Methods

In the period 24 March 2023 – 21 June 2024, we surveyed nine sampling plots with the help of 54 pitfall traps (6 traps in each site). Periods of sampling were: 24.III–14.

VI.2023, 14.VI–6.X.2023, 6.X.2023–23.II.2024 and 23.II–21.VI.2024. The traps were made out of cut 2 L plastic bottles buried at the level of the ground surface and filled with 8% formaldehyde. Additionally, we conducted observations and manual collecting of material. Information about the sampling sites is presented in Table 1.

We conducted several ecological analyses of the species and their communities. The life forms are following the classification of Sharova (1981). According to their hind wing development, species were classified into three groups: macropterous (always possessing wings), wing dimorphic/polymorphic (only part of the population being fully winged), and brachypterous (short-winged/wingless) (Den Boer et al. 1980). According to their ecological requirements in terms of humidity, the recorded carabid species were divided into six categories: hygrophilous, mesohygrophilous, mesophilous, mesoxerophilous, xerobionts and eurybionts. According to their frequency of occurrence (F), the species were divided into euconstant ($F = 100\%$), constant ($F > 50\%$), auxiliary ($F = 25\text{--}50\%$) and accompanying ($F < 25\%$). It was calculated for each species using the formula (1).

$$F = p \cdot P^{-1} \cdot 100, \%, \quad (1)$$

where: p is the number of the sites where the species occur (no matter of its abundance), and P is the number of all studied sites, i.e. $P = 9$.

The dominance structure was calculated on the basis of the classification of Tischler (1949) for soil invertebrates, modified by Sharova (1981): eudominants (with degree of dominance over 10%), dominants (5 to 10%), subdominants (2 to 5%), recedents (1 to 2%) and subrecedents (<1%).

For the mathematical processing of the

Table 1. List and description of the sampling sites.

Site	Location/Description	GPS coordinates	Altitude, m a.s.l.
I	Golak Hill, SE Vetren Dol village. Dry stony ridge meadow with single Jerusalem thorn (<i>Paliurus spina-christi</i> Mill.), oriental hornbeam (<i>Carpinus orientalis</i> Mill.), dog rose (<i>Rosa</i> spp.) and juniper bushes and tufts. Subjected to intensive grazing.	42°08'38" N 24°10'23" E	410
II	Golak Hill, SE Vetren Dol village. Meadow bordered by a gully and a dry stony slope with Jerusalem thorn to the north, and small hornbeam forest to the south. Heavily contaminated with domestic waste, also used as a shooting range.	42°08'46" N 24°10'33" E	326
III	Karkariya Ridge, S Vetren Dol village. Moist gully and a brook in preserved steep European beech (<i>Fagus sylvatica</i> L.) forest, with wild cherry (<i>Prunus avium</i> L.), wych elm (<i>Ulmus glabra</i> Huds.), <i>Anemone</i> sp., nettle (<i>Urtica dioica</i> L.) and dewberry (<i>Rubus caesius</i> L.). At the beginning of the conifer belt.	42°07'25" N 24°09'03" E	813
IV	Karkariya Ridge, S Vetren Dol village. Mixed deciduous forest (beech, oaks, hornbeam, aspen (<i>Populus tremula</i> L.), scattered birch (<i>Betula pendula</i> Roth) and maple (<i>Acer</i> spp.) trees), with dog rose and <i>Luzula</i> sp.	42°07'47" N 24°09'22" E	770
V	Karkariya Ridge, S Vetren Dol village. Oak forest with ivy (<i>Hedera helix</i> L.) and single oriental hornbeam and cornelian cherry (<i>Cornus mas</i> L.). Almost destroyed by logging in the autumn of 2023.	42°07'57" N 24°09'46" E	775
VI	Karkariya Ridge, S Vetren Dol village. Dry oriental hornbeam scrub.	42°08'34" N 24°09'39" E	525
VII	Golak Hill, SE Vetren Dol village. Dry stony slope with oriental hornbeam shrubs and scattered oaks.	42°08'37" N 24°09'54" E	490
VIII	Karkariya Ridge, S Vetren Dol village. Large thermo-mesohygrophilic evergreen glade (with underground mineral waters) along a small river and bordering a forest. Cows' watering place.	42°08'36" N 24°09'19" E	413
IX	Golak Hill, SE Vetren Dol village. Relatively moist north-facing stony slope with scattered hornbeam, dog rose and juniper bushes, mosses and Poaceae grasses; with more soil.	42°09'15" N 24°09'39" E	300

data, MS Excel and the software products PAST, PRIMER6 and TWINSpan were used. The analyses were performed with the data based on the species' dynamic density, calculated as the number of individuals of a given species caught, divided

by the effective (realised) number of trap-days (t.d.) multiplied by 100 (number of individuals of each species captured per 100 t.d.).

Specimens were identified by the first author and are stored at IBER – BAS.

Results and Discussion

During our research, a total of 3184 specimens were collected in the pitfall traps and further 45 were caught by hand. We found 92 carabid species, of which five were collected only by handpicking, so they are not included in the mathematical processing of the data. These were: *Acu-palpus interstitialis* Reitter, 1884, *Agonum emarginatum* Gyllenhal, 1827, *Amara proxima* Putzeys, 1866, *Bembidion deletum* Audinet-Serville, 1821 and *Cicindela campestris* Linnaeus, 1758. The full list of the pitfall-trapped species, along with their authors and years of description, arranged according to their TWINSpan classification, is presented in the Appendix A (Table A1). The list of all manually collected species is given in the Appendix A (Table A2).

Taxonomy and communities

The tribe Harpalini had 29 of the species (31.5 %), and the tribe Amarini was represented by 13 species (14 %). Pterostichini had 10 species (11 %); Carabini and Lebiini had eight species each; Sphodrini had seven; Bembidiini and Brachinini had four; Nebriini, Platynini and Licinini had two; Cicindelini, Notiophilini and Trechini included only one species each. The most species-rich genera were *Harpalus* (17 sp., 18.5 % of all), *Amara* (10 sp., 11 %) and *Carabus* (5 sp., 5 %). The same species share in the genera, with very similar percentages, was also found in steppe habitats in Western Bulgaria, where the tribes Harpalini and Amarini were the most numerous, as well (33 % and 17 %, respectively) (Teofilova and Kodzhabashev 2025). The most common and abundant members of the carabid communities in the studied region were the eudominants

Calathus distinguendus and *Carabus convexus*, and the dominants *Carabus montivagus* and *Laemostenus cimmerius*. There were five subdominants (*Calathus cinctus*, *C. fuscipes*, *Carabus coriaceus*, *Tapinopterus balcanicus* and *Trechus quadristriatus*), three recedents (*Molops dilatatus*, *Myas chalybaeus* and *Nebria brevicollis*) and 75 subrecedents (all others). Of the eudominants, *C. convexus* was also an euconstant species, collected in all seven sampling sites ($F = 100\%$), and *C. distinguendus* was not found in one site only (the moist gully in the beech forest). The first species is quite polytopic (e.g. Turin et al. 2003), while the later was considered diagnostic for the steppe habitats (Teofilova and Kodzhabashev 2025). The other constant species ($F > 50\%$) were *Amara aenea*, *Brachinus expulso*, *Calathus fuscipes*, *C. cinctus*, *Carabus coriaceus*, *Harpalus pumilus*, *H. rufipes*, *H. tardus*, *Laemostenus cimmerius*, *Myas chalybaeus* and *Trechus quadristriatus*. Nineteen species were auxiliary and 55 were accompanying.

Constant species found in the carabid complex during all four sampling periods were: *Abax ovalis*, *Brachinus brevicollis*, *Calathus distinguendus*, *C. fuscipes*, *C. cinctus*, *Carabus montivagus*, *C. coriaceus*, *C. convexus*, *Laemostenus cimmerius*, *Myas chalybaeus*, *Pterostichus oblongopunctatus*, *Tapinopterus balcanicus* and *Trechus quadristriatus*, which probably reflects their higher ecological plasticity.

Zoogeographical patterns

Beetles belonged to 22 categories from the five main faunal types of Bulgarian carabids (Fig. 1): European-Asiatic steppe complex (30 species, 33 %), Mediterranean (*sensu lato*) (21 sp., 23 %), North-

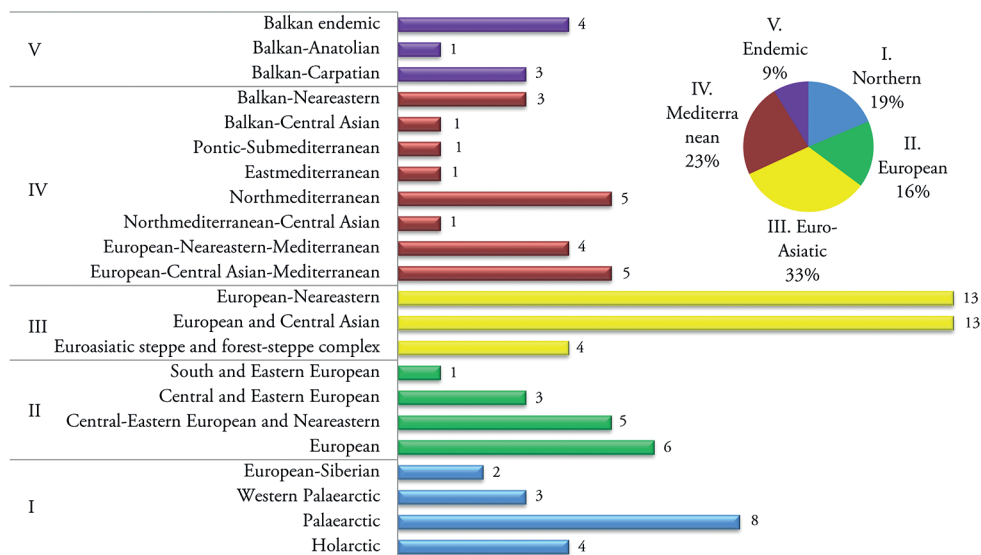


Fig. 1. Zoogeographical categories and types found in the studied carabid complex.

ern Holarctic European-Siberian (17 sp., 19 %), European (15 sp., 16 %) and Endemic (8 sp., 9 %). Most represented were the European-Neareastern and European-Central Asian zoogeographical elements (13 sp., 14 % each).

The distribution of the zoogeographic complexes is consistent with the theory of their origin and subsequent adaptation to xerophytic landscapes appeared after the withdrawal of Paratethys waters (Kryzhanovskij 1983). The Mediterranean and Steppe complexes (>50 % of the species) share a common history but differ in their adaptive specialisation, which is also discussed in the context of their modern distribution, the synanthropisation of habitats and climatic changes that have resulted in a large degree of overlap in their ranges (Kryzhanovskij 2002). The representatives of the Euro-Asiatic group are mostly *Harpalus* and *Amara* species, which predominate both taxonomically and zoogeographically. They are mainly open-living xerobionts, adapted to the extremes of

temperature and humidity values and amplitudes characteristic of temperate steppe zones. The Mediterranean group is also xerobiontic, but the species are thermophilic, given their mostly subtropical range. The predominance of these two zoogeographic complexes in the study area is the result of two main factors – climate and presence of massive karst terrain with sparse vegetation. These complexes prevailed in the xerothermophilous region of the Eastern Rhodopes, too (Teofilova and Kodzhabashev 2020). The other two major complexes include nemoral European and circumpolar Holarctic taxa, which are mostly adapted to inhabit mesophilous and mesohygrophilous forest and riparian habitats (Carabini, Pterostichini, Bembidini).

Life forms

From the two established classes of life forms, class Zoophagous contained 49 species (54 %) and Mixophytophagous

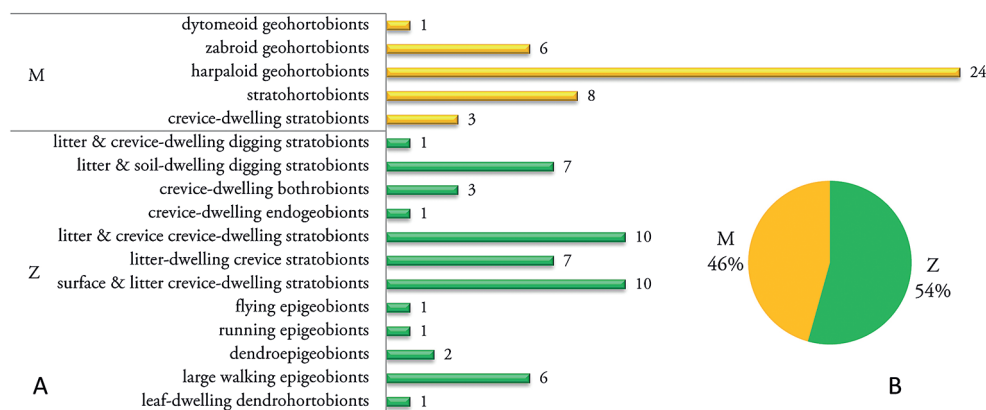


Fig. 2. Life forms of the ground beetles from the studied habitats: A) Number of species belonging to each life form category; B) Share of the two classes.

Note: Z – Zoophaga, M – Myxophytophaga.

were 42 (46 %) (Fig. 2), which according to Sharova (1981) is very close to the typical ratio for the meadow steppes from the Forest-steppe zone (57 %: 43 %) and the semidesert zone of Eurasia (58 %: 42 %). In Bulgaria, the same ratio was established in pseudomauis habitats in the lower belts of the Pirin Mts. (Teofilova 2020b) and in unvegetated or sparsely vegetated inland cliffs, rock pavements and outcrops (Teofilova 2019). Quite similar were the values from surveyed inland screes (56: 44) (Teofilova 2019), different habitats in the Eastern Rhodope Mts. (56.5: 43.5) (Teofilova and Kodzhabashev 2020), and from the urbanised region of the city and surroundings of Plovdiv (57: 43) (Teofilova 2022b). Similar number and share of the groups of life forms found (12 zoophagous: 4 mixophytophagous) was reported for the Forest-steppe zone of Eurasia (Sharova 1981). The most numerous were the harpaloid geohortobionts, which easily move along the stems of herbaceous plants and quickly bury themselves in the topsoil if necessary. Most of these species are from the tribes

Harpalini and Amarini and are specific to open and arid pastures and synanthropic coenoses, both natural and semi-natural.

Wing morphology and humidity preferences

The analysis of the degree of hind wing development of carabids from the study area showed that macropterous were 51 species (57 %), di(poly)morphic or pteridimorphic (some individuals have fully developed wings, others only vestigial ones) were 21 species (23 %) and brachypterous (hind wings shorter than elytra, or missing) were 18 species (20 %) (Fig. 3A). The relatively low number of winged species is comparable only with typical montane habitats, and such (56 % macropterous, 25 % brachypterous and 19 % pteridimorphic) was also found in the preserved steppe habitats in Western Bulgaria (Teofilova and Kodzhabashev 2025). In previously studied mountains, the ratios between macropterous, pteridimorphic and brachypterous species were 57 %, 22 % and 16 % in the Sarnena Sredna Gora

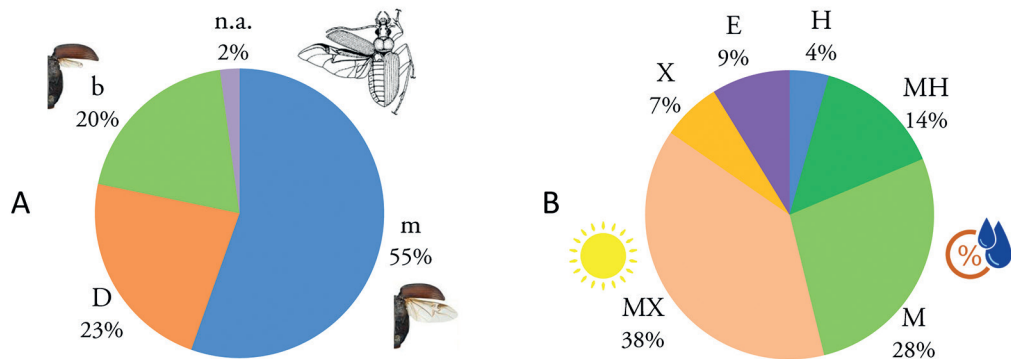


Fig. 3. Wing morphology (A) and humidity preferences (B) of the ground beetles.

Note: m – macropterous, D – di(poly)morphic, b – brachypterous; H – hygrophilous, MH – mesohygrophilous, M – mesophilous, MX – mesoxerophilous, X – xerophilous, E – eurybiont.

Mts. (Teofilova and Kodzhabashev 2021), and 45 %, 32 % and 23 % in a house yard in another part of the Western Rhodope Mts. (Teofilova 2022a). As a comparison, these values were 67 %, 21 % and 6 % in the region of the city of Plovdiv (Teofilova 2022b). According to Sharova (1981), wing reduction can be viewed as an adaptation in two major directions: foraging on slow-moving prey that does not require migrations by flight (as in *Carabus*), or living in the soil, burrows and subterranean cavities, where a robust chitinous armour develops instead of flight wings. Wingless species form stable local populations with limited or reduced dispersal function. Among the myxophytophagous group, winglessness is relatively rare, since they need to move by flight from one food plant to another (Sharova 1981). Most of the harpaloid geochorto- and stratothorobionts found in our study are winged.

In relation to their humidity preferences, mesoxerophilous carabids had the largest share (35 species, 38 % of all). Mesophilous were 25 species (28 %), 13 species (14 %) were mesohygrophilous; eurybiontic group included 8 species

(9 %); xerophilous were 6 species (7 %) and hygrophilous were 4 species (4 %) (Fig. 3B). In the Sarnena Gora Mts., we found a similar situation – mesoxerophilous species were 34 % of all, mesophilous were 27 %, mesohygrophilous were 17 %; strictly hygrophilous (9 %), xerophilous (6 %), and eurybiontic species (7 %) were less represented (Teofilova and Kodzhabashev 2021). Our results are also in accordance with the data from another part of the mountain, where the mesophilous complex prevailed (Teofilova 2022a). Larger was the share of the mesoxerophilous and xerophilous species, for instance, in the carabid complexes in the Eastern Rhodopes (Teofilova and Kodzhabashev 2020), from the relatively dry regions of the city and surroundings of Plovdiv (Teofilova 2022b), and in karstic steppes in Western Bulgaria (Teofilova and Kodzhabashev 2025).

Analysis of α - and β -diversity and spatial distribution

The analysis showed several patterns in the distribution of carabid fauna and

carabid coenoses, as well as the strong degradation of pasture habitats. The greatest number of species was found in the site VIII, the evergreen glade with underground mineral waters, followed by site I, the stony ridge meadow. Both sites are also characterised by the highest values of ecological diversity and the smallest degree of dominance (Table 2). The combination of rich species composition with relatively low dynamic densities and high dominance characterises the 'ecological catastrophe' effect found in habitats with the highest grazing intensity, particularly in sites I and VIII, and to a lesser extent in sites II and IX. In the first two, the herbaceous vegetation is heavily overgrazed and trampled by the extremely large numbers of cattle concentrated on the relatively small karst terrain. The long-term overgrazing has led to severe vegetation degradation, soil erosion and depletion of herbaceous food resources, which also explains the reduction in total biomass of consumers from different food chain levels. The high degree of evenness combined with high values of species rich-

ness and low values of dominance degree are indicative of both stable communities and those in catastrophe, but for different reasons. The disturbed dominance structure in the studied grasslands has led to a levelling of the significance of species with extremely low dynamic densities, which is indicative of the catastrophic effect in four of the studied sites – I, VIII, IX, II (ranked by the degree of anthropogenic influence). In these habitats, despite the use of the dynamic density (instead of absolute abundances) in order to avoid incorrect comparisons, due to the destruction of a large proportion of traps by the large ruminants, we found the effect of lowered quantitative values. In the studied forest habitats, we observed patterns related to the forest type, moisture gradient, location and exposure. Species composition in these ecosystems was limited and the dominant species were relatively large, mesophilic and predominantly predatory. Stable old-growth forests (especially site III) maintain specific fauna and the presence of old trees and dead wood (forming 'old-age islands') is highly supporting for

Table 2. Species richness parameters calculated with PAST software.

Indices	I	II	III	IV	V	VI	VII	VIII	IX
Number of species (S)	33	30	23	16	21	21	29	42	23
Individuals (N)	137	355	329	506	217	758	405	321	156
Dynamic density (DD)	4.98	14.07	14.90	22.33	9.81	35.87	16.42	16.55	5.40
Dominance (D)	0.121	0.268	0.156	0.308	0.184	0.363	0.209	0.112	0.313
Simpson (1-D)	0.879	0.732	0.844	0.692	0.816	0.637	0.791	0.888	0.687
Shannon (H)	2.752	1.985	2.369	1.532	2.246	1.493	2.140	2.833	1.915
Evenness (e^H/S)	0.475	0.243	0.465	0.289	0.450	0.212	0.293	0.405	0.295
Brillouin	2.444	1.865	2.252	1.480	2.096	1.449	2.031	2.639	1.725
Menhinick	2.819	1.592	1.268	0.711	1.426	0.763	1.441	2.344	1.841
Margalef	6.504	4.939	3.796	2.409	3.718	3.016	4.664	7.104	4.357
Equitability (J)	0.787	0.584	0.755	0.552	0.738	0.490	0.635	0.758	0.611
Fisher (alpha)	13.800	7.818	5.631	3.145	5.740	4.000	7.154	12.910	7.446
Chao-1	39.60	33.60	30.50	19.00	26.00	24.75	36.20	49.86	38.00

Note: The highest values are given in **bold**.

peculiar carabid coenoses consisting of mostly stenotopic and endemic species, such as *Molops alpestris*, *M. dilatatus*, *Myas chalybaeus*, *Tapinopterus balcanicus* and *Xenion ignitum*. Dynamic densities ranged from 15 to 35 ind. per 100 t.d., which indirectly reflects the productive capacity of the ecosystem. The values in site VI indicated an ecotone effect at the forest-grassland interface, manifested by strongly increased dynamic density com-

bined with low evenness and negligible species diversity.

The mathematical processing showed some valuable objectives. Both the clustering and the TWINSpan classification detached the sites with forest habitats in a separate group (Fig. 4 and Table A1).

The TWINSpan method is a hierarchical classification approach that allows for levels of analysis. It classifies both samples and species, providing maximum

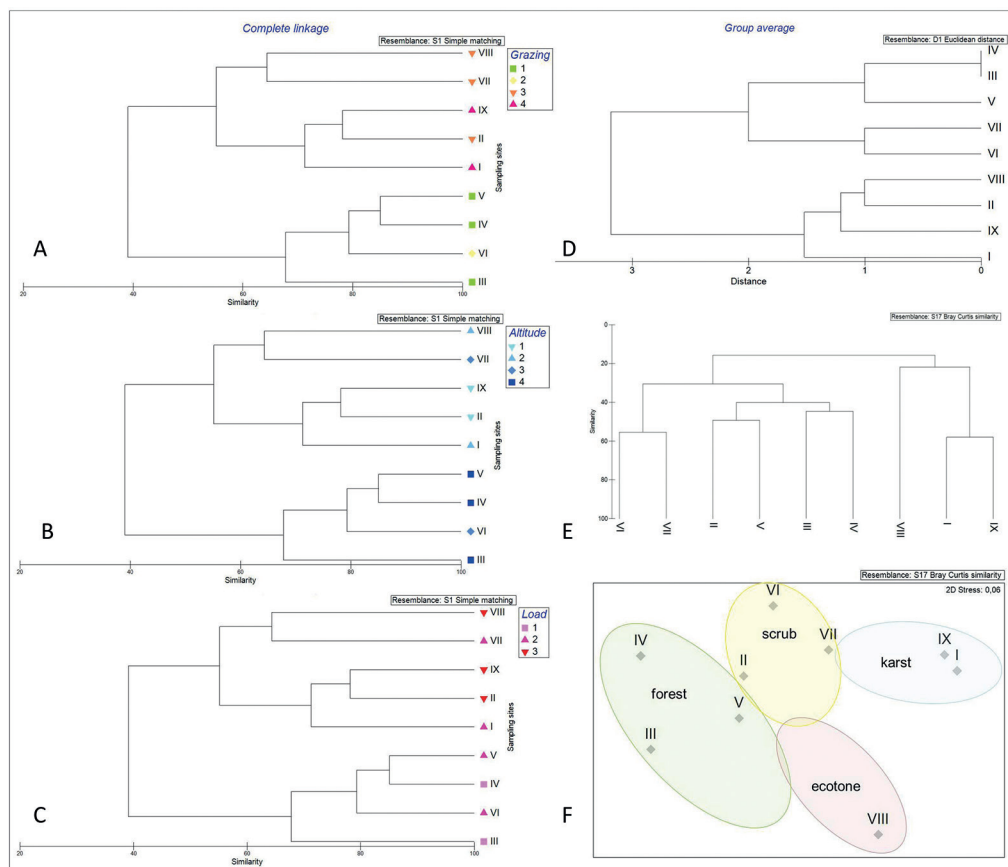


Fig. 4. Mathematical analyses based on the carabid dynamic density across nine sampling sites and in dependence of different factors.

Note: A) Simple matching based on the level of cattle grazing intensity (1 – low/none, 2 – medium, 3 – strong, 4 – overgrazed); B) Simple matching by altitude (1 – 300 to 326 m a.s.l., 2 – 410 to 413 m, 3 – 490 to 525 m, 4 – 770 to 813 m); C) Simple matching by anthropogenic load, e.g. human presence or trash accumulation (1 – low, 2 – medium, 3 – strong); D) Euclidean distance between sites; E) Group average similarity; F) Multidimensional scaling (MDS).

opportunities for ecological interpretation and visualisation of the distribution of all species along the existing gradients represented in the input data. In our study, TWINSpan showed that the separation of the forest habitats (level 1) was based on the indicator species *Carabus montivagus* and *Tapinopterus balcanicus*. Non-preferential species for this level were: *Amara aenea*, *Calathus distinguendus*, *C. fuscipes*, *Carabus coriaceus*, *C. convexus*, *Harpalus rufipes*, *Laemostenus cimmerius*, *Myas chalybaeus* and *Trechus quadristriatus*. All of them with the exception of *M. chalybaeus* were in the 'negative' (*0), non-forest group of species. On level 2, the specific and species-rich habitat VIII was separated, with *Amara eurynota* as an indicator. The similarity of the forest habitats was also confirmed by the Euclidean distance between the sites (Fig. 4D). The complete linkage analysis conducted with simple matching clustering showed that of the three gradients set, the degree of grazing was the most significant and the overgrazed habitats formed a well-defined group (Fig. 4A). The impact of grazing on the number of invertebrates has been previously proved and it concerns the soil structure in pastures (deteriorated from the intense trampling by the grazing animals), the grassland litter, plant species diversity and dry matter density (see Duffey 1975, Byers et al. 2000, Suominen et al. 2003, Aleksandrowicz and Bagińska 2009, Bassett 2009, Toupet et al. 2020, Schwerk et al. 2021). Pastures located on eroded karst terrains, such as the studied, are subject to extremely high human impact, where grazing must be carefully planned, monitored and, if necessary, corrected. These habitats are strategic for the survival of many animal and plant species, but both the cessation of grazing and the overgrazing and overtrampling

are detrimental to their existence. It was found that grazing and restoration treatment did not have detectable effects on carabid abundance, biomass, or diversity, but carabid communities responded differently to grazing within different grassland types (Waite et al. 2022). Grazing alone seemed to have no effect on carabid abundance in the study of Bassett (2009), as well. It has even been suggested that moderate levels of cattle grazing might have positive effects on carabid communities (Suominen et al. 2003, Waite et al. 2022). However, there is a proved relation between the grazing intensity and carabid assemblage structure, and variables such as timing, duration, cessation and intensity of grazing have to be taken into account (Schwerk et al. 2021).

Furthermore, beetle diversity and abundance are positively correlated with plant biomass, and since grazing reduces plant biomass, plant height, and plant structure, we could conclude that it will reduce beetle diversity and abundance, especially if it is heavy and consistent (Bassett 2009). Based on our results, we could clearly state that the grazing in the study area has led to severe degradation and ecological catastrophe effect, which requires the immediate intervention of the authorities to regulate the number of grazing animals and the duration of the grazing and exclusion periods. Given the global climate changes and prolonged summer droughts, the rationing and regulation of grazing must consider the need of sufficient food resources for all animal species of the pastoral complex, among which there are some strictly protected or key species (potential prey of rare birds and mammals).

The altitude had a similar effect as the grazing (Fig. 4B), since the most actively grazed habitats were situated at the lower

altitudes. Along with the grazing, the elevation has been pointed out as the most important predictor of carabid abundance, biomass, species richness and diversity, which had a significant effect on carabid diversity with higher diversity in upper elevations (Bassett 2009). Similar was also the effect of the anthropogenic load, mostly connected with waste disposal, human presence and tourism (Fig. 4C). We could also note, that in addition to the habitat type separation, the ecotone edge effect and ecological catastrophic effect were both present, as well (Fig. 4F), as it was also indicated by the analysis of the species richness parameters (Table 2). It should not be overlooked that all rare carabid species from the typical pastoral complex are represented by single specimens, which is an important indicator of the state of the environment. The biomass of the collected beetles is negligible, which is a function of the productivity of the ecosystem.

The analysis of the ecological condition in the studied habitats, carried out using faunistic and ecological parameters of the ground beetles, showed a strong dependence of the environmental gradients on both natural factors and those created by the human presence and activities. The taxonomic, zoogeographic and ecological structures analysed showed the extremely high degree of indicativeness, suitable to be used when necessary to establish the ecological state and level of anthropogenic impact, for ecological monitoring purposes.

Conclusion

The main motivation for this study was the request of the local people to protect the surroundings of their settlement from large and severe encroachments of investment intentions. All results were analysed us-

ing as a model group the ground beetles, which are good indicators of both ecological conditions and anthropogenic changes.

The ecological and zoogeographical structures showed that the carabid fauna in the study region represents unique combination of steppe, xerothermophilous and montane elements. Both qualitative and quantitative results demonstrated the separation of the forest habitats and clearly pointed that the grazing in the area of Golak Hill has led to severe ecological degradation and ecological catastrophe effect.

Constantly changing environmental conditions require periodic monitoring of biotic parameters to provide information on the current status as well as to predict future trends, which could support proper maintenance of priority grassland, forest and riparian habitats. The analyses carried out here with the help of the carabids have proved to be extremely practical, accurate and applicable for ecological monitoring purposes when it is necessary to develop a restoration programme for habitats affected by human activities and when justifying proposals for the protection of a particular area.

The area of study has extremely high conservation significance and we give some practical recommendations for its conservation and preservation of food resources and habitats of all animal species of the pastoral complex (including protected or key species):

- Pastures require strict, constant control of the number and type of grazing animals and rationing of grazing periods;
- There is a need for creating isolated patches of scrub and woody vegetation and a peripheral forest 'inviolability' zone where grazing is completely restricted and prohibited;
- In forest habitats it is necessary to isolate areas with groups of large, old

trees;

- Global trends of warming, droughting and summer aridity should be reconsidered, and systems for channeling, collecting and storing of rainwater for use in grazing livestock and maintaining grazing habitats should be built as a precaution;
- The area should be protected and a management plan should be developed with legislated long-term strategies, regimes and management and funding mechanisms.

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Appendix A

Table A1. TWINSpan classification of the sampling sites and pitfall-trapped species, and indicator species for each level of division.

Species	Sampling sites										Species group code
	VIII	I	II	VII	IX	VI	III	IV	V		
<i>Calathus fuscipes</i> (Goeze, 1777)	5	1	-	4	3	5	-	2	2	*000	
<i>Amara aenea</i> (De Geer, 1774)	4	2	2	2	2	-	1	1	-	*0010	
<i>Harpalus distinguendus</i> (Duftschmid, 1812)	2	1	1	-	-	-	-	-	-	*00110	
<i>Harpalus signaticornis</i> (Duftschmid, 1812)	2	1	-	-	1	-	-	-	-	*00110	
<i>Leistus rufomarginatus</i> (Duftschmid, 1812)	2	-	-	2	-	-	-	-	-	*00110	
<i>Ophonus cribricollis</i> (Dejean, 1829)	2	-	2	2	-	-	-	-	-	*00110	
<i>Calosoma sycophanta</i> (Linnaeus, 1758)	1	-	1	-	-	1	-	-	-	*001110	
<i>Amara anthobia</i> A.Villa & G.B. Villa, 1833	3	1	-	-	-	-	-	-	-	*001111	
<i>Amara convexior</i> Stephens, 1828	2	-	-	-	-	1	-	-	-	*001111	
<i>Amara lucida</i> (Duftschmid, 1812)	3	1	-	-	-	-	-	-	-	*001111	
<i>Amara similata</i> (Gyllenhal, 1810)	1	-	-	-	-	-	-	-	-	*001111	
<i>Asaphidion flavipes</i> (Linnaeus, 1760)	2	-	-	-	-	-	-	-	-	*001111	
<i>Bembidion dalmatinum</i> Dejean, 1831	2	-	-	-	-	-	-	-	-	*001111	
<i>Harpalus caspius</i> (Steven, 1806)	3	-	-	1	-	-	-	-	-	*001111	
<i>Harpalus rubripes</i> (Duftschmid, 1812)	2	-	-	1	-	-	-	-	-	*001111	
<i>Harpalus saxicola</i> Dejean, 1829	4	-	-	1	-	-	-	-	-	*001111	
<i>Nebria brevicollis</i> (Fabricius, 1792)	5	-	-	-	-	-	-	-	-	*001111	
<i>Parophonus maculicornis</i> (Duftschmid, 1812)	2	-	-	-	-	-	-	-	-	*001111	
<i>Parophonus mendax</i> (P.Rossi, 1790)	2	-	-	-	-	-	-	-	-	*001111	
<i>Pterostichus nigrita nigrita</i> (Paykull, 1790)	1	-	-	-	-	-	-	-	-	*001111	
<i>Stenolophus teutonius</i> (Schränk, 1781)	1	-	-	-	-	-	-	-	-	*001111	
<i>Syntomus obscuroguttatus</i> (Duftschmid, 1812)	1	-	-	-	-	-	-	-	-	*001111	
<i>Syntomus pallipes</i> (Dejean, 1825)	3	-	-	-	-	-	-	-	-	*001111	
<i>Zabrus spinipes</i> (Fabricius, 1798)	3	-	2	-	-	-	-	-	-	*001111	
<i>Amara saphyrea</i> Dejean, 1828	-	-	1	-	1	1	-	-	-	*010000	
<i>Calosoma inquisitor</i> (Linnaeus, 1758)	-	-	-	-	-	2	-	-	-	*010000	
<i>Harpalus picipennis</i> (Duftschmid, 1812)	-	-	-	-	-	1	-	-	-	*010000	
<i>Acinopus picipes</i> (Olivier, 1795)	-	2	2	-	2	-	-	-	-	*010001	
<i>Acinopus ammophilus</i> Dejean, 1829	-	-	2	-	-	-	-	-	-	*010001	
<i>Amara equestris</i> (Duftschmid, 1812)	-	3	2	-	-	-	-	-	-	*010001	
<i>Brachinus brevicollis</i> Motschulsky, 1844	1	1	5	-	1	-	-	-	-	*010001	
<i>Brachinus crepitans</i> (Linnaeus, 1758)	-	-	2	-	-	-	-	-	-	*010001	
<i>Calathus ambiguus</i> (Paykull, 1790)	-	3	-	-	-	-	-	-	-	*010001	
<i>Cymindis axillaris</i> (Fabricius, 1794)	-	3	1	2	2	-	-	-	-	*010001	
<i>Cymindis lineata</i> (Quensel, 1806)	-	2	-	1	2	-	-	-	-	*010001	
<i>Dixus obscurus</i> (Dejean, 1825)	-	2	2	-	-	-	-	-	-	*010001	
<i>Harpalus attenuatus</i> Stephens, 1828	-	1	-	2	-	-	-	-	-	*010001	

Species	Sampling sites									Species group code
	VIII	I	II	VII	IX	VI	III	IV	V	
<i>Harpalus flavicornis</i> Dejean, 1829	-	-	2	-	-	-	-	-	-	*010001
<i>Harpalus pumilus</i> Sturm, 1818	1	2	2	2	2	-	-	-	-	*010001
<i>Harpalus smaragdinus</i> (Duftschmid, 1812)	-	1	-	-	-	-	-	-	-	*010001
<i>Harpalus taciturnus</i> Dejean, 1829	-	2	-	2	-	-	-	-	-	*010001
<i>Laemostenus</i> sp. (probably new)	-	1	2	-	-	-	-	-	-	*010001
<i>Lebia humeralis</i> Dejean, 1825	-	1	-	-	-	-	-	-	-	*010001
<i>Licinus cassideus</i> (Fabricius, 1792)	-	2	-	-	-	-	-	-	-	*010001
<i>Licinus silphoides</i> (P.Rossi, 1790)	-	2	1	-	1	-	-	-	-	*010001
<i>Microlestes fissuralis</i> (Reitter, 1901)	-	1	-	1	-	-	-	-	-	*010001
<i>Microlestes minutulus</i> (Goeze, 1777)	-	-	-	1	-	-	-	-	-	*010001
<i>Ophonus sabulicola</i> (Panzer, 1796)	-	-	-	4	-	-	-	-	-	*010001
<i>Philorhizus notatus</i> (Stephens, 1827)	-	2	-	2	-	-	-	-	-	*010001
<i>Zabrus tenebrioides</i> (Goeze, 1777)	-	-	1	-	-	-	-	-	-	*010001
<i>Brachinus explodens</i> Duftschmid, 1812	2	2	4	2	2	-	-	-	-	*010010
<i>Ophonus azureus</i> (Fabricius, 1775)	1	1	-	3	1	-	-	-	-	*010010
<i>Harpalus serripes serripes</i> (Quensel, 1806)	2	2	2	-	1	-	-	-	-	*010011
<i>Harpalus subcylindricus</i> Dejean, 1829	1	-	-	1	2	-	-	-	-	*010011
? <i>Zabrus rhodopensis</i> Apfelbeck, 1904	2	-	1	1	3	-	-	-	-	*010011
<i>Calathus distinguendus</i> Chaudoir, 1846	4	5	3	5	5	5	-	1	2	*0101
<i>Calathus cinctus</i> Motschulsky, 1850	2	5	5	3	4	2	-	-	3	*0101
<i>Harpalus tardus</i> (Panzer, 1796)	4	2	2	3	1	2	-	-	-	*0101
<i>Carabus coriaceus cerisyi</i> Dejean, 1826	2	3	4	5	3	5	-	2	4	*011
<i>Laemostenus cimmerius</i> (Fischer von Waldheim, 1823)	3	2	5	5	3	5	-	3	5	*011
<i>Carabus convexus dilatatus</i> Dejean, 1826	2	2	5	5	1	5	5	5	5	*100
<i>Myas chalybaeus</i> (Palliard, 1825)	1	2	2	3	-	5	1	4	3	*100
<i>Harpalus pygmaeus</i> Dejean, 1829	-	-	-	1	-	-	-	-	1	*101
<i>Notiophilus rufipes</i> Curtis, 1829	-	-	1	-	-	1	-	1	1	*101
<i>Ophonus laticollis</i> Mannerheim, 1825	-	-	-	-	-	1	1	-	-	*101
<i>Amara eurynota</i> (Panzer, 1796)	1	-	-	-	-	-	-	-	2	*1100
<i>Harpalus rufipes</i> (De Geer, 1774)	2	-	-	-	1	2	2	-	3	*11010
<i>Pterostichus niger</i> (Schaller, 1783)	2	-	-	-	-	-	5	-	-	*11010
<i>Trechus quadristriatus</i> (Schränk, 1781)	5	-	1	3	1	2	5	3	4	*11010
<i>Cychrus semigranosus</i> Palliard, 1825	-	-	-	-	-	2	3	2	-	*11011
<i>Harpalus atratus</i> Latreille, 1804	-	-	-	-	-	2	3	-	-	*11011
<i>Molops alpestris rhilensis</i> Apfelbeck, 1904	-	-	-	-	-	2	-	2	3	*11011
<i>Molops dilatatus dilatatus</i> Chaudoir, 1868	-	-	-	-	-	4	2	4	4	*11011
<i>Abax ovalis</i> (Duftschmid, 1812)	-	-	-	-	-	-	4	-	4	*111
<i>Amara montivaga</i> Sturm, 1825	-	-	-	-	-	-	1	-	1	*111
<i>Anisodactylus binotatus</i> (Fabricius, 1787)	-	-	-	-	-	-	-	-	1	*111
<i>Aptinus bombardia</i> (Illiger, 1800)	-	-	-	-	-	-	4	-	2	*111
<i>Bembidion castaneipenne</i> Jacquelin du Val, 1852	-	-	-	-	-	-	1	-	-	*111

Species	Sampling sites									Species group code
	VIII	I	II	VII	IX	VI	III	IV	V	
<i>Carabus montivagus</i> Palliardi, 1825	-	-	-	-	-	-	4	5	2	*111
<i>Carabus intricatus</i> Linnaeus, 1760	-	-	-	-	-	-	4	3	-	*111
<i>Carabus hortensis</i> Linnaeus, 1758	-	-	-	-	-	-	3	-	1	*111
<i>Laemostenus venustus</i> (Dejean, 1828)	-	-	-	-	-	-	2	-	-	*111
<i>Platynus scrobiculatus purkynei</i> Obenberger, 1917	-	-	-	-	-	-	2	-	-	*111
<i>Pterostichus oblongopunctatus</i> (Fabricius, 1787)	-	-	-	-	-	-	4	-	-	*111
<i>Pterostichus strenuus</i> (Panzer, 1796)	-	-	-	-	-	-	1	-	-	*111
<i>Tapinopterus balcanicus balcanicus</i> Ganglbauer, 1891	-	-	-	-	-	-	5	5	2	*111
<i>Xenion ignitum</i> (Kraatz, 1875)	-	-	-	-	-	-	5	1	-	*111
Habitat group code	*00	*010	*010	*010	*010	*011	*1	*1	*1	
Level 1							<i>C. montivagus</i> , <i>T. balcanicus</i>			
Level 2	<i>A. eurynota</i>									
Level 3	<i>A. aenea</i>									

Note: The arrangement of the groups of species and habitats is produced by the TWINSpan software, as well as the denotations (codes) of these groups. The different colouration distinguishes the different groups of habitats at the respective levels.

Table A2. List of the carabids manually collected in the studied region.

Species	Locality and material
<i>Abax ovalis</i> (Duftschmid, 1812)	site V, 1♂, 14.VI.2023
<i>Acinopus picipes</i> (Olivier, 1795)	E Vetren Dol, 42°08'43" N, 24°11'08" E, 262 m, alfalfa, 1♂, 14.VI.2023
<i>Acupalpus interstitialis</i> Reitter, 1884	S Vetren Dol, 42°07'09.3" N, 24°09'07.8" E, 888 m, small waterfall in beech forest, 1♀, 15.VI.2023
<i>Amara aenea</i> (De Geer, 1774)	Golak Hill, 42°08'56" N, 24°09'56" E, 438 m, 1♀, 25.III.2023
<i>Amara anthobia</i> A.Villa & G.Villa, 1833	Vetren Dol, 42°08'47" N, 24°09'09" E, 400 m, 1♂, 25.III.2023
<i>Amara proxima</i> Putzeys, 1866	site II, 1♂, 24.III.2023
<i>Bembidion dalmatinum</i> Dejean, 1831	S Vetren Dol, 42°07'09" N, 24°09'08" E, 888 m, 3♂, 15.VI.2023; site III, 1♀ 2♂, 21.VI.2024
<i>Bembidion deletum</i> Audinet-Serville, 1821	Karkariya Ridge, 42°07'45" N, 24°09'46" E, 790 m, 1♀, 15.VI.2023
<i>Calathus fuscipes</i> (Goeze, 1777)	SE Vetren Dol, 42°08'38" N, 24°10'46" E, 276 m, 1♀ 1♂, 15.VI.2023
<i>Carabus intricatus</i> Linnaeus, 1760	SE Vetren Dol, 42°07'54" N, 24°10'15" E, 612 m, 1 ex., 26.III.2023; site III, 1 ex., 22.VI.2024
<i>Carabus coriaceus cerisyi</i> Dejean, 1826	SE Vetren Dol, 42°08'38" N, 24°10'46" E, 276 m, 1 ex., 15.VI.2023
<i>Cicindela campestris</i> Linnaeus, 1758	top of the Golak Hill, 1 ex., 25.III.2023; S Vetren Dol, 42°07'22" N, 24°08'37" E, 1020 m, oak forest, 1 ex., 15.VI.2023
<i>Cymindis lineata</i> (Quensel, 1806)	Golak Hill, 42°08'50" N, 24°09'42" E, 400 m, 2♀ 1♂, 14.VI.2023
<i>Dixus obscurus</i> (Dejean, 1825)	S Vetren Dol, 42°08'49" N, 24°09'23" E, 440 m, pine-oak ecotone, 1♀, 13.VI.2023; top of Golak Hill, 42°08'50" N, 24°09'42" E, 400 m, 1♂, 14.VI.2023
<i>Harpalus caspius</i> (Steven, 1806)	S Vetren Dol, 42°08'49" N, 24°09'23" E, 440 m, 1♀, 15.VI.2023
<i>Harpalus taciturnus</i> Dejean, 1829	site II, 1♂, 24.III.2023
<i>Molops dilatatus</i> Chaudoir, 1868	Karkariya Ridge, 42°07'45" N, 24°09'46" E, 790 m, 1♀, 15.VI.2023
<i>Notiophilus rufipes</i> Curtis, 1829	site V, 1♀, 21.VI.2024
<i>Platynus scrobiculatus purkynei</i> Obenberger, 1917	S Vetren Dol, about 42°07'19" N, 24°09'07" E, 870 m, near brook in beech forest, 1♀ 1♂, 24.III.2023
<i>Pterostichus nigrita</i> (Paykull, 1790)	site III, 3♀, 21.VI.2024
<i>Trechus quadristriatus</i> (Schränk, 1781)	Karkariya Ridge, 42°06'56" N, 24°08'53" E, 1074 m, beech forest, 1♀, 15.VI.2023
<i>Xenion ignitum</i> (Kraatz, 1875)	Karkariya Ridge, 42°06'56" N, 24°08'53" E, 1074 m, 1♂, 15.VI.2023

Assessment of soil properties related to soil health in spruce forests of Pirin National Park

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Thematic field: Ecosystem biodiversity, conservation and environmental restoration

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Abstract

Forest soil is a key component of mountain ecosystems, providing water storage, nutrient cycling, carbon sequestration, and biodiversity support. This study assessed essential physical and chemical parameters related to the health status of Cambisols in mature Norway spruce (*Picea abies* L.) stands within Pirin National Park, Southwestern Bulgaria. Ten soil profiles were sampled across an altitudinal range of 1386–1790 m a.s.l. Soil depth varied between 30 and 67 cm, according to WRB (2022) and regional classification criteria, all were considered shallow, potentially limiting root development and water storage capacity. In the A horizon, pH_{H₂O} ranged from 4.1 (very strongly acidic) to 6.2 (slightly acidic), while CEC was high to very high (11.96–33.52 cmol(+)·kg⁻¹), largely dominated by exchangeable Ca²⁺. Base saturation varied from 61 % to 99 % in surface horizons, but unsaturated conditions (<50 % BS) occurred in 50 % of the Bw horizons. Organic carbon content in the A horizon ranged from 25.81 to 63.36 g·kg⁻¹, with C/N ratios from 8 to 15, and available water holding capacity from 32 % to 68 %. These results indicate that although the soils are well supplied with organic matter and have favourable porosity and bulk density, acidity, low base saturation in some profiles, and dominance of chemically inactive texture fractions (sand and silt) may constrain soil health. The findings provide a regional baseline for soil health assessment in Pirin National Park, with practical implications for sustainable forest management and conservation planning in other mountainous protected areas.

Key words: basic soil parameters, Cambisols, forest soils, soil organic carbon, soil pH.

Introduction

Forest ecosystems cover about 31 % of the Earth's land area and provide essen-

tial ecosystem services, including carbon sequestration, water filtration, and biodiversity support (FAO 2020). At the foundation of these services is soil health, which

reflects the soil's capacity to sustain biological productivity, maintain environmental quality, and promote plant health (Doran and Zeiss 2000). In 1910, Wallace introduces the term 'soil health' for first time, referring to soil fertility (Wallace 1910, Brevik 2018). Over the years the meaning of the term has expanded. In the early 90s it was defined by Doran and Parkin (1994), as the 'continued capacity of soil to function as a vital living system'. Soil health is a multidimensional concept encompassing the biological, physical, and chemical properties of soil that influence its ability to support plant growth, regulate water, cycle nutrients, and sustain ecosystem functions (Doran and Zeiss 2000, Lehmann et al. 2020, Janzen et al. 2021, Friedrichsen et al. 2021). It is increasingly recognised as a key factor in ensuring sustainable agricultural systems, contributing to food security, and mitigating climate change (Lal 2001, 2009). Soil health is a fundamental determinant of ecosystem productivity, particularly in forest environments, where it regulates nutrient cycling, water retention, and the support of biodiversity.

Forest soils are vital for the long-term sustainability of forest ecosystems, influencing tree growth, forest resilience to disturbances, and the overall functioning of the biosphere (Schlesinger 1997). In forested landscapes, soil health is not only critical for plant productivity but also for carbon sequestration, soil biodiversity, and maintaining ecosystem services (Jobbágy and Jackson 2000). However, forest soils face several challenges, including deforestation, soil compaction, nutrient depletion, and the impact of climate change, which can significantly degrade soil health (Binkley and Fisher 2013). In many forest regions it is under threat due to land-use change, unsustainable forestry, pollution, etc. (Pan et al. 2011). There-

fore, it is crucial to assess soil health in forest areas. Assessing forest soil health requires an integrated approach using indicators such as SOM, microbial biomass, enzyme activity, bulk density, and nutrient status (Karlen et al. 2003). Monitoring and understanding the current status of forest soil health is crucial for mitigating degradation and promoting ecosystem resilience. Long-term soil monitoring networks, such as those in Europe (ICP Forests) and North America (FIA), are essential for detecting trends and guiding policy (de Vries et al. 2003).

For agricultural soils there are several scales and indexes that were introduced to assess soil health, but for the forest soils there is still a lack of information regarding unified indicators for its assessment (DeLuca et al. 2019). Therefore, the main aim of our study was to assess key soil parameters related to soil health status in forest areas of Pirin National Park, Southwestern Bulgaria.

Materials and Methods

Study area

The study was conducted in mature pure stands of Norway spruce (*Picea abies* (L.) Karst.) within Pirin National Park, Southwestern Bulgaria. The approximately central coordinates of the park are 41°44'33.8" N, 23°25'49.7" E. Ten soil profiles were dug up in spruce stands, covering an elevation gradient from 1386 to 1790 m a.s.l. (Table 1).

The focus of this study are forest soils, developed mostly on acidic igneous, and metamorphic rocks. There were also finite areas with limestones of the sedimentary rocks. The soil forming rocks are mainly granites. These rocks are composed of

able 1. Location and main properties of the studied soil profiles

Soil profile (SP)	Coordinates	Altitude, m a.s.l.	Exposition	Soil unit
1	41°46'16.13" N, 23°25'39.24" E	1790	SE	Eutric Cambisols
2	41°47'03.76" N, 23°26'49.71" E	1698	E	Eutric Cambisols
3	41°46'53.48" N, 23°26'50.97" E	1711	E	Dystic Cambisols
4	41°47'10.23" N, 23°27'08.60" E	1640	NW	Eutric Cambisols
5	41°46'53.48" N, 23°26'50.97" E	1719	NE	Eutric Cambisols
6	41°46'21.0" N, 23°27'34.70" E	1660	NE	Dystic Cambisols
7	41°46'26.11" N, 23°27'33.08" E	1653	NE	Dystic Cambisols
8	41°46'53.14" N, 23°26'37.03" E	1678	NW	Dystic Cambisols
9	41°46'59.06" N, 23°27'20.02" E	1643	NE	Dystic Cambisols
10	41°47'52.03" N, 23°28'40.01" E	1386	NE	Eutric Cambisols

large quantises of free quartz, which is a prerequisite for acidic reaction of the soils (Halder 2020). The metamorphic rocks are marbled limestones, and gneisses. The studied soils were classified as Dystic Cambisols and Eutric Cambisols (WRB 2022). The relief of the studied area is typically mountainous. Pirin Mountain is a horst-block high-mountain structure, part of the so-called Rila-Rhodope massif. It is characterised by steep slopes and deep river valleys. Pirin Mountain is in the Continental-Mediterranean climate zone. In the middle part of the mountain average summer temperatures ranges between 14 °C and 16 °C. At an altitude of 1600 m the average monthly summer temperatures in July and August are around 20 °C. The average winter temperatures vary between -2 and -5 °C. Absolute minima of the air temperature in the winter was -31 °C. Annual precipitations in the lower altitudes of the park vary between 600–700 mm and up to 1000–1200 mm in the highest altitudes (Velev 2002).

Soil sampling and analyses

Ten representative spruce stands were selected for setting the soil profiles on the

territory of Pirin National Park. To have comparability of the results regarding the indicative parameters for soil health assessment, were selected matured seed pure spruce stands. Litter samples were taken as one pooled sample (LFH). Soil samples were taken from each mineral horizon of the studied soil profiles for chemical analyses. Steel cylinders were used to determine bulk density. All soil analyses were carried out in the Central laboratory at the University of Forestry.

To assess key soil properties related to soil health status, 14 indicators were selected – soil depth, $\text{pH}_{\text{H}_2\text{O}}$, $\text{pH}_{\text{CaCl}_2}$, exchangeable acidity, bulk density, particle density, soil porosity, soil texture, org. C, total N, org. C/N ratio, water holding capacity (available soil water content), cation exchange capacity and base saturation. These indicators were determined for the mineral horizons of the profiles.

The following methods were employed for the analysis of the soil samples:

- soil pH (H_2O and CaCl_2) – ISO 10390:2005 (ISO 2005);
- exchangeable acidity, determined according to ISO 14254:2018 using BaCl_2 (ISO 2018b);
- bulk density – determined in accor-

dance with ISO 11272:1998 (ISO 1998);

- particle density – measured by pycnometer method, according to ISO 787-10:1993 (ISO 1993).

Soil porosity was calculated using equation (1):

$$\text{Soil porosity} = \left(1 - \frac{\text{Bulk density}}{\text{Particle density}} \right) \cdot 100, \% \quad (1)$$

The soil texture was determined in accordance with ISO 11277:2009 (ISO 2009). Humus and soil organic carbon contents were assessed using the modified Turin method (120 °C, 45 min with CuSO_4 as catalyst) as described by Kononova (1963), and Filcheva and Tsadilas (2002). Total nitrogen content was measured by using Kjeldal's method with KjeltexAuto 1030 Analyser. Exchangeable cations (Ca^{2+} , Mg^{2+} , K^+ , Na^+) were measured in accordance with ISO 11260:2018 (ISO 2018a). Available soil water holding capacity was assessed using the Kachinskii method (Kachinskii 1963). Bulk density was determined according to ISO 11272:1998 (ISO 1998).

Results and Discussion

Assessment of basic physical and chemical parameters related to soil health status in the studied spruce forests include indicators such as pH, soil organic matter (SOM), soil texture, bulk density, porosity, available water holding capacity, nutrients, etc. It should be noted that forest soils differ significantly from agricultural soils (Paltineanu et al. 2020). Relationships between woody vegetation and soil in forest ecosystems are complex. Their interactions are more dynamic and competitive regarding the soil nutrients and water supply (Trogisch et al. 2021). In order to assess soil health in its entirety and

interrelationships with forest vegetation, it is required a long period of time for monitoring and assessment of complex soil parameters. In the present study, basic soil parameters were studied and assessed to establish soil health status of specific soil unit.

Physical properties of the soil

Soil depth directly impacts the rooting depth of forest vegetation. Deep soils provide ample space for root systems, enabling trees to access water and nutrients from larger volumes of soil (Canadell et al. 1996). Shallow soils limit root development, reducing a tree's anchorage and increasing susceptibility to windthrow and drought stress. Soil depth strongly influences a forests ability to retain and supply water during dry periods. Deeper soils have higher total water holding capacity, which is crucial for sustaining transpiration and photosynthesis during drought events (Leuschner et al. 2001).

All the studied soils were shallow. The soil depth of the soil profiles varied between 30 and 64 cm. The A horizon was assessed as shallow. It ranges from 5 cm up to 23 cm. In most of the profiles Bw horizon was also shallow. The soil depth in this horizon was between 18 cm and 50 cm.

Soil texture is a key parameter which affects water holding capacity, aeration, and root penetration. Loamy soils are generally ideal for tree growth due to their balanced properties. However, texture also influences other parameters such as bulk density and moisture dynamics (Gee and Bauder 1986).

The obtained results for A and Bw horizons of the studied forest soils are shown on figures 1 and 2.

The soil texture in A horizon of the

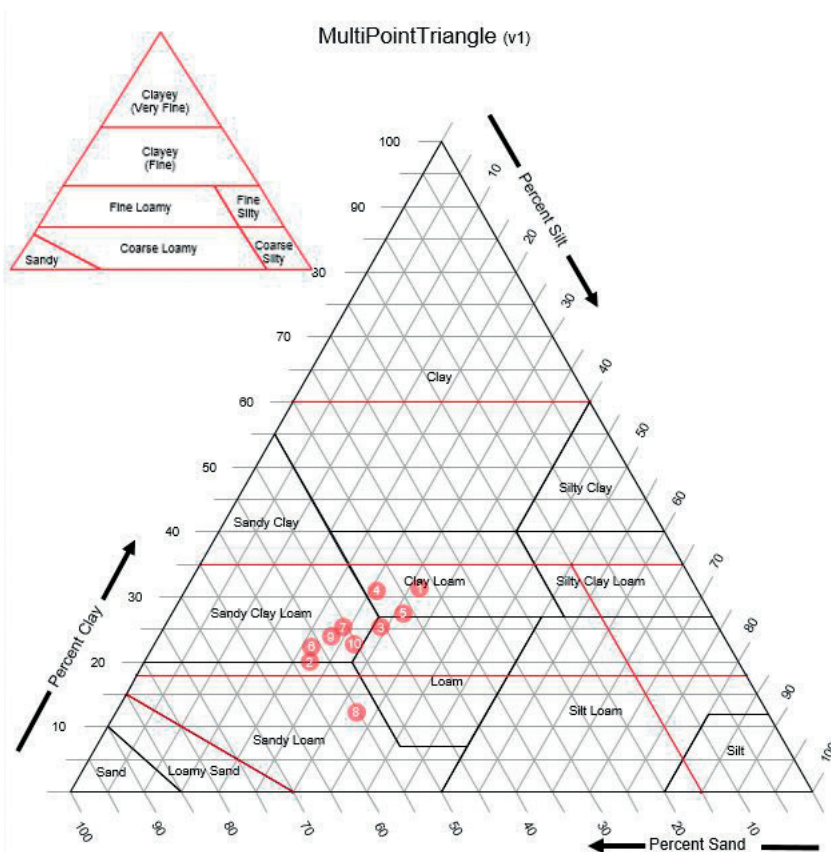


Fig. 1. Soil texture in A horizon of the studied soils.

studied Cambisols varies in the category of loamy soils as follows: sandy clay loam (SP 2, 6, 7, 9, and 10), clay loam (SP 1, 4, and 5), sandy loam (SP 8) and loam (SP 3). It was found that in A horizon the predominant fraction for most of the soil profiles is sand. Its value varies in the range from 36.19 % to 57.77 %. Silt fraction is in the second place with relatively high values, which varies in the range from 21.38 % (SP 6) to 32.38 % (SP 8). The higher percentage content of the silt fraction in A horizons negatively affects the soil health of these soils, since silt is a chemically inactive part of the fine soil and does not provide high amounts of nu-

trient plant uptake. It is a well-known fact that silty soils have deteriorated general physical properties and do not provide good conditions for the growth and development of fine roots of woody vegetation. Clay fraction has the lowest values in the range from 12.26 % (SP 8) to 31.26 % (SP 1). It should be noted that the clay content in the surface horizon in one of the soil profiles is over 30 % (SP 1). Based on the obtained results of the other soil samples, it can be assumed that this amount is not due to a clay formation process in situ. It is a result of the increased organic colloids in the soil samples due to the higher humus content (7.72 % in SP 1). The higher

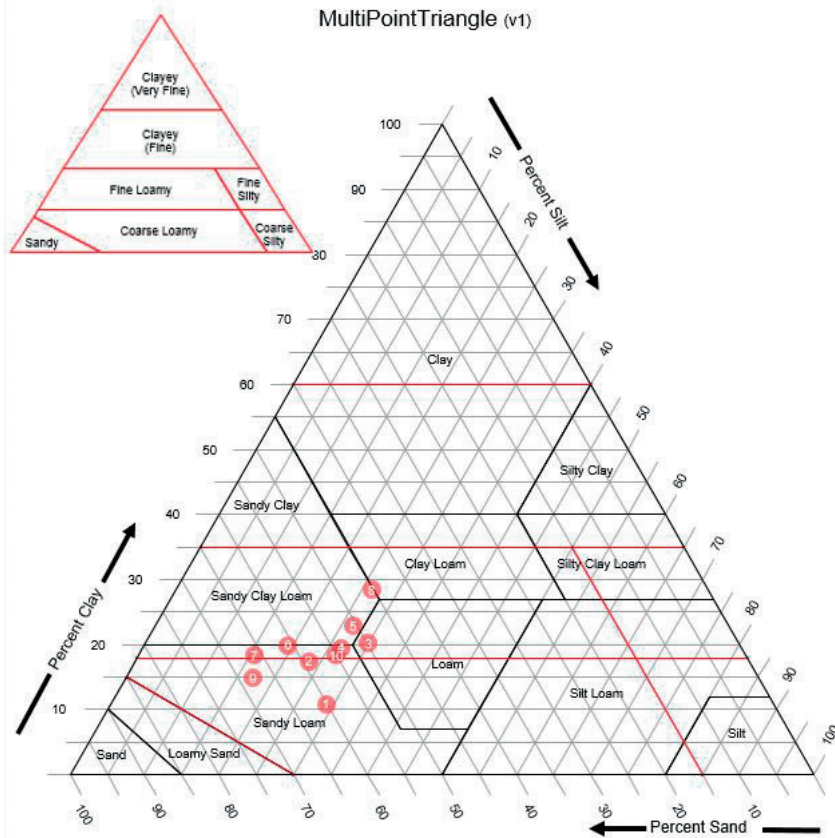


Fig. 2. Soil texture in Bw horizon of the studied soils.

amounts of sand and silt determine the lower values of the bulk density of the soil and the higher values of porosity in this horizon. The soil texture is dominated by chemically low-active fractions.

In Bw horizon, the soil texture is also dominated by sand and silt. It varies in the category of loamy soils (Fig. 2). In 70 % of the studied samples, it is assessed as sandy loam (SP 1, 2, 4, 6, 9, and 10). In 20 % it is sandy clay loam (SP 5 and 8). And in 10 % of cases, it is loam (SP 3). The sand content varies from 45.25 % (SP 8) to 67.98 % (SP 9). The content of silt is between 15.57 % (SP 7) and 29.98 % (SP 3). The clay varies between 10.84 %

(SP 1) and 28.42 % (SP 8). Petrova et al. (2024) reported similar results for Cambisols developing under the influence of spruce forests.

The results obtained for the bulk density in A horizon show that it varies between $0.68 \text{ g}\cdot\text{cm}^{-3}$ and $0.97 \text{ g}\cdot\text{cm}^{-3}$ (Fig. 3). It is assessed as ideal and easy for the root penetration of the woody vegetation and germination of seeds. This estimate also favours the healthy status of the soils, since the water, air and temperature regime of the soil directly depend on the values of the bulk density. In the meta-morphic Bw horizon, the bulk density of the soil ranges from $0.96 \text{ g}\cdot\text{cm}^{-3}$ (SP 7)

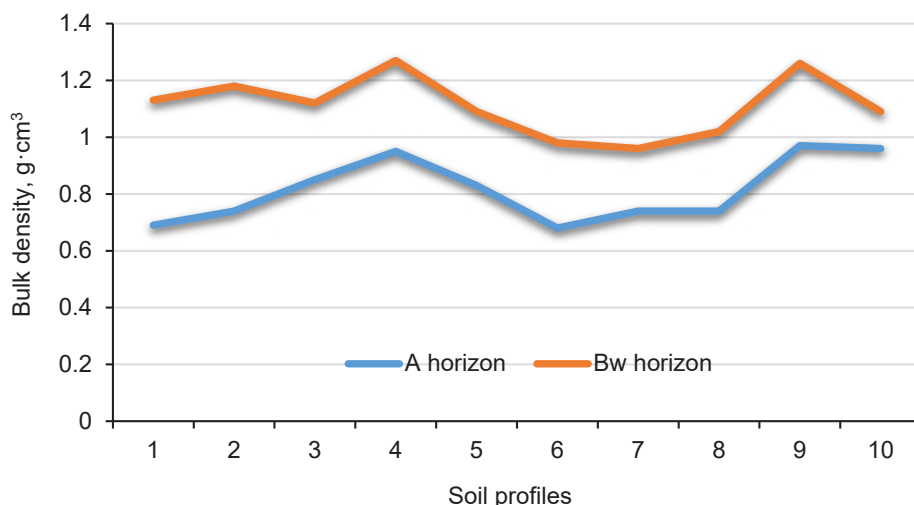


Fig. 3. Soil bulk density.

and $1.27 \text{ g}\cdot\text{cm}^{-3}$ (SP 4) and is assessed as loose to compacted.

The values of the particle density are shown on Figure 4. In A horizon it varies in the range of 2.30 (SP 3) up to 2.51 (SP 4). The lower values are due to the significant amount of SOM in the soil compared to the Bw horizon where the mineral part

predominates. In Bw horizon the values are higher because of the larger amount of quartz in it.

The porosity of the soils was also assessed. For A horizon it varies from 57% (SP 10) up to 71% (SP 1 and 2). It was over 50% for all soil profiles, which is assessed as very good porosity. In the depth

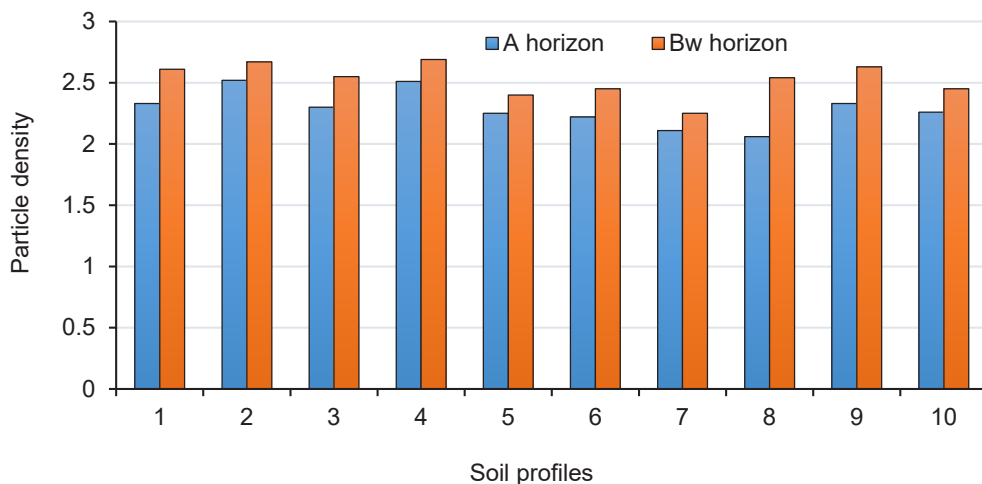


Fig. 4. Soil particle density.

of the soil the values of porosity naturally decrease, however it should be noted that in the metamorphic horizon the porosity is high and is also assessed as very good. It ranges from 52 % (SP 9) up to 60 % (SP 6 and 8).

Basic soil chemical properties

Soil pH influences nutrient availability, microbial activity, and root development. Most forest soils maintain a slightly acidic to neutral pH (between 5.5 and 7.0), which optimizes nutrient uptake for most tree species (Brady and Weil 2016). De-

viations can result in nutrient deficiencies or toxicities, particularly of aluminium and manganese in acidic soils. Acidification can lead to the leaching of essential nutrients such as calcium and magnesium, which are critical for forest health. Additionally, changes in nutrient cycling, driven by soil acidification, can affect the availability of nutrients for forest trees (Iwald et al. 2013).

In the studied soils we have obtained soil pH for forest litter and mineral horizons of the soil profiles. The results are presented in Table 2.

The reaction of LFH layer varies in the

Table 2. Soil reaction in the studied soils.

Soil profile (SP)	Layer/ Horizon	Depth, cm	pH _{H2O}	pH _{CaCl2}	Exchangeable acidity, cmol(+)·kg ⁻¹
1	LFH	1–0	5.5	5.2	0.57
	A	0–20	6.2	5.9	0.38
	Bw	20–60↓	7.1	6.6	0.15
2	LFH	1–0	5.4	5.1	0.89
	A	0–5	4.6	4.0	2.40
	Bw	5–30↓	4.8	4.2	1.48
3	LFH	2–0	5.5	5.2	0.90
	A	0–23	5.4	4.6	0.91
	Bw	23–62↓	5.0	4.0	4.32
4	LFH	2–0	5.5	5.2	0.98
	A	0–20	5.4	4.7	0.61
	Bw	20–38	5.1	4.5	0.81
	BC	38–50↓	5.1	4.5	0.66
5	LFH	1–0	5.1	4.7	1.13
	A	0–12	4.5	3.7	7.30
	Bw	12–30	4.7	4.0	3.39
	BC	30–60↓	4.7	3.9	2.76
6	LFH	1–0	4.9	4.2	1.10
	A	0–11	4.3	3.4	4.69
	Bw	11–43↓	4.0	3.5	5.56
7	LFH	1–0	4.7	4.3	0.98
	A	0–8	4.1	3.4	5.13
	Bw	8–40	4.0	3.4	6.66
8	LFH	1–0	5.8	5.3	0.73
	A	0–5	4.8	4.1	1.64
	Bw	5–55↓	4.4	3.9	3.10

Soil profile (SP)	Layer/Horizon	Depth, cm	pH _{H2O}	pH _{CaCl2}	Exchangeable acidity, cmol(+)-kg ⁻¹
9	LFH	1–0	5.3	4.8	0.72
	A	0–8	4.5	4.0	3.16
	Bw	8–32	4.9	4.3	2.10
	C	32–64↓	5.4	4.8	1.10
10	LFH	1–0	5.5	5.1	0.86
	A	0–6	5.8	5.3	0.45
	Bw	6–42↓	4.8	4.2	2.22

range of 4.7 to 5.8, which is assessed as very strongly acidic to moderately acidic (Table 2). The average pH_{H2O} of the litter is 5.4, which is assessed as moderately acidic. The exchangeable acidity values in this layer vary from 0.57 to 1.10 cmol(+)-kg⁻¹, which are assessed as low (Vanmechelen et al. 1997). Assessing the reaction of aboveground litterfall is important because it can contribute to the acidification of soils. The lower values of litter pH have significant effects on the mineral part of forest soils. Acidic litter, such as those from conifers forests may lower soil pH, potentially affecting nutrient availability and microbial activity (Augusto et al. 2002). In A horizon of the studied Cambisols, the soil reaction is assessed as very strongly acidic (SP 5, 6, 7, and 9) to neutral (SP 1). The obtained low pH values are a prerequisite for the occurrence of destructive processes in the soil and deterioration of soil health. It should be noted that the average soil depth of A horizon is 12 cm. The acidic reaction of the spruce litterfall has a significant impact on the values of the active soil reaction in it. In the depth of the soil, pH_{H2O} is maintained in the acidic spectrum. An exception is SP 1, in which it is neutral. It is assessed as very strongly acidic (SP 5, 6, 7, and 8) to neutral (SP 1). The results obtained for Bw horizon show that their values are relatively close to those in A horizon. This indicates that in some of the profiles in

the depth of the soil, soil acidification processes are taking place. Petrova (2018) pointed out that low pH values pose a great risk to soil health and can lead to the initial phase of the podzolisation process in some regions of the country. Exchangeable acidity is also a very important indicator of soil health. Its values were measured potentiometrically (pH in CaCl₂ solution) and titrimetrically (extraction with BaCl₂). The pH values in CaCl₂ were used to assess the soil's buffer capacity, following Ulrich scale (1983). The obtained results showed that the buffer capacity of the soil in 30 % of cases in the A horizon is due to the weathering of secondary silicates (clay minerals) and the release of exchangeable aluminum (SP 2, 8, and 9). The soil in the humus-accumulative horizon of these profiles has a high buffer capacity (Ganev 1990). In another 30 % of cases in very strongly acidic soils – pH < 3.8, the consumption of protons is due to the dissolution of iron oxides (SP 5, 6, and 7). And in this pH range, the buffer capacity of the soils is low. In 20 % the most intensive process is the exchange of protons with basic cations – Ca²⁺ and Mg²⁺, which are adsorbed exchangeably on the surface of the colloidal structures and in this pH interval the buffer capacity of the soils is low (SP 3 and 4). In the other 20 % of cases the buffering is due primarily to the weathering of primary silicates in the soil (SP 1 and 10). The buffer capacity of

the soil in these cases is also low.

As for Bw horizon, the predominant process in 50 % of the studied soil profiles is weathering of secondary silicates and the buffer capacity is estimated as high (SP 2, 3, 5, 8, and 10). In 20 % of the cases, the most intensive process is the exchange of protons with basic cations and the buffer capacity of the soils is low (SP 4 and 9). For 20 % of the studied profiles, the buffer capacity of the soil in the metamorphic horizon is due to the dissolution of iron oxides (SP 6 and 7). The buffer capacity of the soil in this horizon is low. Only in 10 % of the cases in SP 1, the buffer capacity of the soil is realised mainly by the dissolution of calcium carbonate. The buffer capacity of this soil is high.

The obtained values for titrimetrically measured exchangeable acidity are shown in Table 2. For the studied metamorphic soils, it was found that in A horizon the exchangeable acidity varies in a wide range from very low (SP 1 and 10) to high (SP 5 and 7). In Bw horizon the exchangeable acidity values are assessed as very low (SP 1) to high (SP 7). The high exchangeable acidity values indicate ongoing destructive processes in the surface horizons of the studied profiles, which negatively affects their health status.

Two of the essential chemical indicators of soil health are cation exchange capacity (CEC) and base saturation (BS). These parameters influence nutrient dynamics, buffering against pH fluctuations, and the soil's capacity to resist degradation (Ulrich 1986, Likens and Bormann 1995, Brady and Weil 2016). Their evaluation is significant for guiding soil fertility management and long-term sustainability.

The obtained results for basic chemical properties of the studied Cambisols are presented in Table 3.

CEC values of the litter vary in the range

between $48.86 \text{ cmol}(+)\cdot\text{kg}^{-1}$ (SP 2) and $68.56 \text{ cmol}(+)\cdot\text{kg}^{-1}$ (SP 8). It is assessed as very high. The litterfall is highly saturated with bases between 98 % and 99 %. The surface horizon is characterised with high CEC values. It varies significantly from $11.96 \text{ cmol}(+)\cdot\text{kg}^{-1}$ to $33.52 \text{ cmol}(+)\cdot\text{kg}^{-1}$ and is assessed as high to very high. In A horizon the high CEC values are due to higher content of exchangeable calcium. BS of soil is also high. It varies from 61 % to 99 % and is assessed as high to very high. For the metamorphic horizon CEC ranges from $3.03 \text{ cmol}(+)\cdot\text{kg}^{-1}$ (SP 3) to $19.80 \text{ cmol}(+)\cdot\text{kg}^{-1}$ (SP 1) and is assessed as low to high.

The BS values vary in a very wide range from 16 % to 99 %. The soil is unsaturated with bases in SP 3, 6, 7, 8, and 9. In profiles 1, 2, 4, 5, and 10, it is saturated with bases.

Soil organic matter – humus content, org. C and org. C/N ratio

SOM is a key component of forest soil health, influencing water retention, nutrient availability, and microbial activity (Six et al. 2002). Forest soils typically have high SOM due to litter input and minimal disturbances. However, several studies (Williams 2003, Lal 2005, FAO 2020) pointed out a decline in SOM in areas experiencing deforestation or logging without organic residue return. For example, intensive cuttings in temperate forests in North America and Europe has led to significant losses in soil carbon stocks (Johnson and Curtis 2001), which affects directly soil health status. In our study the soil profiles were set in undisturbed area of Pirin National Park. The results for SOM and content of Total N (TN) are presented in Table 4.

The content of org. C in the litter of

Table 3. Exchangeable bases, cation exchange capacity (CEC) and base saturation (BS).

Soil profile (SP)	Layer/ Horizon	Depth, cm	Exch. acidity	Exch. Ca	Exch. Mg	Exch. K	Exch. Na	Sum of Exch. bases	CEC	BS %
cmol(+)·kg ⁻¹										
1	LFH	1–0	0.57	44.77	10.24	1.62	0.06	56.69	57.26	99
	A	0–20	0.38	25.74	6.91	0.45	0.05	33.14	33.52	99
	Bw	20–60↓	0.15	15.50	4.05	0.09	0.01	19.65	19.80	99
2	LFH	1–0	0.89	39.69	5.71	2.50	0.07	47.97	48.86	98
	A	0–5	2.40	8.10	1.23	0.19	0.03	9.56	11.96	80
	Bw	5–30↓	1.48	2.53	0.68	0.08	0.02	3.32	4.80	69
3	LFH	2–0	0.90	48.19	7.36	2.66	0.08	58.29	59.18	98
	A	0–23	0.91	12.11	3.43	0.22	0.20	15.96	16.87	95
	Bw	23–62↓	4.32	2.21	0.83	0.08	0.37	3.50	7.82	45
4	LFH	2–0	0.98	48.67	7.64	2.65	0.17	59.13	60.11	98
	A	0–20	0.61	12.46	3.11	0.12	0.05	15.74	16.35	96
	Bw	20–38	0.81	3.16	1.38	0.05	0.03	4.62	5.43	85
	BC	38–50↓	0.66	3.00	1.35	0.04	0.03	4.43	5.09	87
5	LFH	1–0	1.13	47.23	3.50	1.66	0.06	52.45	53.58	98
	A	0–12	7.30	9.71	1.27	0.23	0.03	11.24	18.54	61
	Bw	12–30	3.39	4.06	0.40	0.17	0.03	4.65	8.04	58
	BC	30–60↓	2.76	0.80	0.24	0.07	0.02	1.14	3.90	29
6	LFH	1–0	1.10	41.35	4.39	2.83	0.10	48.66	49.76	98
	A	0–11	4.69	8.62	0.95	0.21	0.06	9.84	14.53	68
	Bw	11–43↓	5.56	0.79	0.13	0.07	0.06	1.05	6.60	16
7	LFH	1–0	0.98	46.59	4.11	1.67	0.08	52.46	53.45	98
	A	0–8	5.13	10.73	0.91	0.24	0.05	11.94	17.07	70
	Bw	8–40	6.66	2.15	0.25	0.14	0.06	2.60	9.26	28
8	LFH	1–0	0.73	51.61	4.86	2.37	0.08	58.92	59.65	99
	A	0–5	1.64	26.17	2.45	0.43	0.05	29.10	30.74	95
	Bw	5–55↓	3.10	1.14	0.29	0.09	0.05	1.57	4.67	34
9	LFH	1–0	0.72	43.39	4.05	1.51	0.09	49.03	49.75	99
	A	0–8	3.16	8.75	0.88	0.24	0.07	9.93	13.08	76
	Bw	8–32	2.10	0.67	0.07	0.07	0.11	0.92	3.03	31
	C	32–64↓	1.10	0.99	0.16	0.05	0.44	1.64	2.74	60
10	LFH	1–0	0.86	53.10	6.77	1.40	0.08	61.36	62.22	99
	A	0–6	0.45	23.04	2.37	0.32	0.06	25.79	26.24	98
	Bw	6–42↓	2.22	2.46	0.57	0.06	0.05	3.14	5.36	59

the studied forest soils varies between 329.04 g·kg⁻¹ (SP 9) and 525.86 g·kg⁻¹ (SP 5). It is assessed as medium to very high (Vanmechelen et al. 1997).

In A horizon, the content of org. C is

in the range from 25.81 g·kg⁻¹ (SP 4) to 63.36 g·kg⁻¹ (SP 7). It is assessed as medium to high, with higher values being formed at higher altitudes. For the metamorphic horizon, the values of org. C are

Table 4. Soil organic matter, total N and org. C/N ratio.

Soil profile (SP)	Altitude, m	Layer/ Horizon	Depth, cm	Humus	Org. C g·kg ⁻¹	Total N	C/N
1	1790	LFH	1–0		396.48	12.48	32
		A	0–20	77.18	44.71	3.57	13
		Bw	20–60↓	20.51	11.89	1.04	11
2	1698	LFH	1–0		399.90	11.83	34
		A	0–5	45.48	26.42	2.34	11
		Bw	5–30↓	12.35	7.14	0.62	11
3	1711	LFH	2–0		401.27	12.56	32
		A	0–23	59.22	34.32	2.31	15
		Bw	23–62↓	20.33	11.81	0.75	16
4	1640	LFH	2–0		415.93	12.17	34
		A	0–20	44.48	25.81	2.13	12
		Bw	20–38	9.83	5.68	0.45	13
		BC	38–50↓	6.11	3.56	0.30	12
5	1719	LFH	1–0		525.86	13.18	40
		A	0–12	76.92	44.61	4.10	11
		Bw	12–30	41.94	24.35	1.73	14
		BC	30–60↓	12.26	7.11	0.56	13
6	1660	LFH	1–0		406.54	14.69	28
		A	0–11	94.85	55.03	6.40	9
		Bw	11–43↓	59.79	34.73	2.46	14
7	1653	LFH	1–0		428.99	14.93	29
		A	0–8	109.12	63.36	6.27	10
		Bw	8–40	85.70	49.73	4.21	12
8	1678	LFH	1–0		390.78	12.14	32
		A	0–5	101.68	59.04	6.99	8
		Bw	5–55↓	21.99	12.78	1.02	13
9	1643	LFH	1–0		329.04	13.04	25
		A	0–8	78.86	45.73	3.78	12
		Bw	8–32	35.34	20.53	0.92	22
		C	32–64↓	10.48	6.11	0.44	14
10	1386	LFH	1–0		382.50	12.34	31
		A	0–6	75.39	43.73	3.17	14
		Bw	6–42↓	25.88	15.04	1.20	13

estimated as very low (5.68 g·kg⁻¹ in SP 4) to high (49.73 g·kg⁻¹ in SP 7). In general, the organic matter content in Bw horizon is estimated as medium.

The amount of total nitrogen (TN) in LFH layer is assessed as low (11.83 g·kg⁻¹ in SP 2) to medium (14.93 g·kg⁻¹ in SP 7).

In A horizon it varies in the range from 2.13 g·kg⁻¹ (SP 4) to 6.99 g·kg⁻¹ (SP 8). It is assessed as low to very high. In the depth of the soil profiles TN values vary from very low (0.45 g·kg⁻¹ in SP 4) to high (4.21 g·kg⁻¹ in SP 7).

The aboveground litter enters the soil

surface with an average (25 in SP 9) to high (40 in SP 5) org. C/N ratio. In most of the litters, it is assessed as average. The transformation of organic matter is an important indicator of soil health, because it provides valuable information regarding the uptake of nitrogen. For mineral horizons of the soil, it decreases. In the surface horizon in all profiles, it is assessed as very low. For Bw horizon it is assessed as very low (11 in SP 1 and 2) to low (22 in SP 9). In summary the studied soils are characterised as average to well stocked with humus and TN.

Available soil water is essential for physiological functions of trees and microbial activity. Forest soils exhibit dynamic water regimes influenced by canopy cover, organic matter, and topography (Bonan 2008). The available water holding capacity (AWHC) of the soil has a significant impact on soil health status of the soils. AWHC is a valuable indicator of soil health due to its close links with ecosystem services such as nutrient availability, biological activity, and resilience to extreme weather. It highly depends on

soil texture and SOM content of the soil. Fine-textured soils such as loams and clays typically have higher AWHC than coarse-textured sandy soils due to greater surface area and microporosity (Rawls et al. 2003).

The obtained data for the studied Cambisols regarding the values of AWHC is presented on Figure 5.

In the surface horizon AWHC values range between average (32 % in SP10) and very high (68 % in profile 6). In Bw horizon, it is between 28 % in SP 2 and 49 % in SP 7. In conclusion, it should be noted that the studied forest soils are average to well stocked with available water due to high amount of humus in the soil. They provide relatively good enough moisture for the fine roots of spruce trees.

Conclusion

The studied forest soils are porous and are characterised by optimal bulk density, which have a positive effect on their health status. These soils are assessed

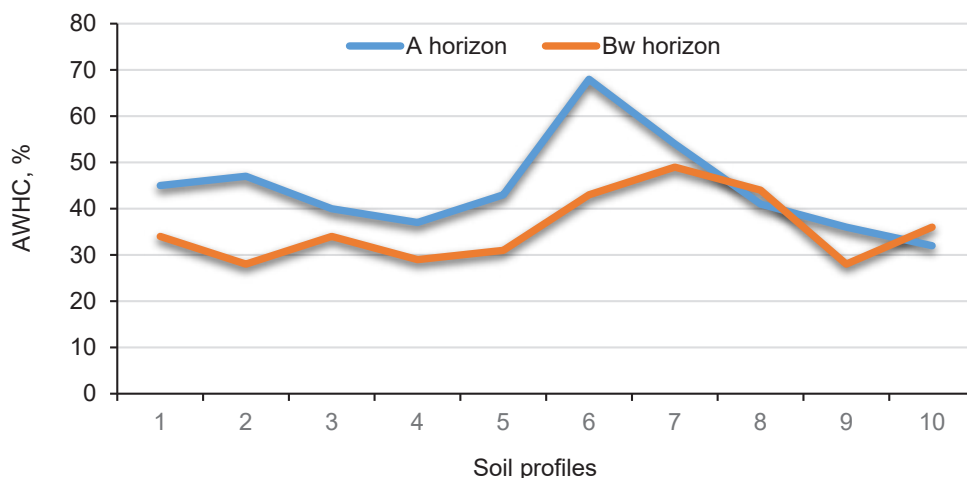


Fig. 5. Available water holding capacity of the soils.

as shallow. The soil texture is dominated by sand and silt fractions which can have a negative impact on soil health, because these fractions are chemically inactive in terms of nutrient uptake of the woody vegetation. These soils are characterised by an acidic reaction, which can lead to acidification processes and loss of organic matter. The buffer capacity in A horizon in most of the soil profiles is high, but in depth of the soil, it is assessed as low. Humus content is assessed as average to high in A horizon. The soils are well stocked with organic matter, and this has a positive effect on plant nutrition, although the org. C/N values are assessed as average. In most of the cases soils are average to well stocked with available water. In conclusion, it can be noted that 50 % of the studied soils provide relatively good conditions for the growth of spruce forest stands and can be assessed as healthy soils.

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