

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

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

This volume of *Forestry Ideas* (vol. 31, No Special Issue 2) presents a second selection of 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.

It complements Special Issue 1 by continuing the publication of scientific contributions presented at the conference, offering further insight into current developments, challenges, and innovations in the field of natural resource management and forest science.

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 second Special Issue of *Forestry Ideas* (vol. 31, No Special Issue 2) with the publication of selected peer-reviewed papers from CENFORKNOW 2025.

Following the strong interest and the high number of quality submissions, this issue builds upon No Special Issue 1 and includes additional articles that broaden the thematic scope of the collection and further reflect the scientific diversity of the event.

This Special Issue has been made possible thanks to the 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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Recovery of herbicide-damaged oilseed rape (*Brassica napus* L.) via biostimulant treatment

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

Thematic field: Agriculture in mountainous and semi-mountainous regions

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Abstract

Oilseed rape (*Brassica napus* L.) is among the most essential oil crops worldwide. The main limiting factor for its growth and development is the uncontrolled weed infestation. Primary method for weed control is the application of herbicides. If applied improperly they can cause herbicidal phytotoxicity. One phenomenon for this stress is the herbicide drift from one field to another. Therefore, the current study aimed to investigate the response of the conventional oilseed rape variety 'PT 225' to simulated herbicide drift and subsequent ameliorative treatment with plant biostimulant. In this regard, herbicidal drift from a field of Clearfield oilseed rape to the conventional one was imitated. The herbicidal product used was Cleranda SC (17.5 g·L⁻¹ imazamox and 375 g·L⁻¹ metazachlor) used for weed management in Clearfield oilseed rapes. The trial was conducted during 2020/2021 and 2021/2022 on the field of the Agricultural University – Plovdiv, Bulgaria. Treatments of the trial were: 1. Untreated control; 2. 0.40 L·ha⁻¹ (20 % of the rate 2.00 L·ha⁻¹); 3. 0.60 L·ha⁻¹ (30 % of the rate 2.00 L·ha⁻¹); 4. 0.40 L·ha⁻¹ + Amino Expert[®] Impuls (selected biostimulant) – 3.00 L·ha⁻¹, and 5. 0.60 L·ha⁻¹ + Amino Expert Impuls – 3.00 L·ha⁻¹. The levels of phytotoxicity were evaluated by the 9-score scale of European Weed Research Society. Parameters as total leaf chlorophyll content, number of primary branches, plant height at the end of the growing season, seed yield, weight of 1000 seeds, and seed oil content were studied. The damages caused by the herbicidal drift were more severely pronounced at 30 % of the solution fallen to the plants. The ameliorative treatment with the plant biostimulant Amino Expert Impuls helped the plants overcome the herbicidal stress, and the plants showed higher results for the studied indicators.

Key words: herbicide drift, herbicide stress, phytotoxicity, plant biostimulant, weed management.

Introduction

According to the data obtained from the FAO (2019), oilseed rape (*Brassica napus* L.) is the second most important oilseed crop around the globe. The oil production from it is considered to be 20 % of the world's total oil production (Hu et al. 2017). The oilseed rape is a source of high-quality protein for animals and to produce biofuel (Raboanatahiry et al. 2018).

For obtaining high and sustainable yields from the oilseed rape, effective weed management is of great importance (Yanev 2020, Tonev et al. 2021). In the conventional agriculture, weed control in different crops is based mainly on chemical herbicidal treatments. The success of modern herbicides is due to their selective action (Cobb and Reade 2010). However, in some cases, they can cause stress to the crop plants or herbicidal phytotoxicity (Tonev and Vassilev 2011). One of the issues that causes herbicidal toxicity is the herbicide drift (Vischetti et al. 2002, Mueller 2015). This is the movement of herbicidal solutions from the target areas to areas that are not intended to be applied. The main factors leading to herbicide drift are spray particle size, application method, boom height, relative humidity and temperature, wind direction, spray pressure, spray angle, etc. (Dexter 1993). Sufficiently high herbicide doses can kill the weeds as well as the crops.

In contrast, the low rates may have no effect on weeds or crops. The action of a separate herbicide is usually determined by its chemical and physical properties (Streibig 2003). Plants which are tolerant to certain herbicides can detoxify it (Cole 1994, Yuan et al. 2007), but the stressed plants receiving wrong herbicide treatment had reduced nitrogen and increased potassium levels in the leaves (Neshev

2020). The stress factors can lead to flowering interruption and decrease the aboveground biomass weight (Huang and Xiong 2009, Takeno 2016, Nesheva and Bozhkova 2021).

If the plants cannot detoxify the herbicidal ingredient to a sufficient level, different functional impairments may be observed in plants. Most of the time, the herbicidal phytotoxicity is chronic, but in some cases, as drift, it can be lethal for the crop. The damage levels may be determined visually or by different physiological and biochemical indicators (Dayan and Zaccaro 2012, Dayan et al. 2015, Neshev et al. 2022). Plant biostimulants can be applied to improve plant growth and development. According to Calvo et al. (2014), a number of reports show the positive effects of biostimulants on plants exposed to different types of stress (biotic or abiotic).

The potential to recover plants that are damaged by herbicides is based on the levels of the structural-functional impairments. As described by Jablonkai (2013), chronic herbicidal toxicity can be overcome (completely or to some extent) by treatments with different products such as plant biostimulants, foliar fertilisers, growth regulators, herbicide antidotes, etc. The foliar treatment with biostimulant to imazamox-damaged maize plants ameliorates the crop's growth, photosynthesis, and crop productivity (Balabanova et al. 2023). An improvement in winter wheat damaged by imazamox after the application of protein hydrolysate was found by Balabanova (2021). Balabanova et al. (2016) also found that a biostimulant treatment considerably restores the physiological status of IMI-R sunflower plants treated with imazamox. After foliar treatment of oats and winter wheat with the herbicides glyphosate, topramezone, and atrazine, Soltani et al. (2015) recorded a

notable increase in yields obtained from the crops due to biostimulants spraying. Neshev et al. (2020) reported a recovery effect on the pumpkin plants after applying imazamox with the biostimulant as an ameliorative treatment.

The information about the effects of biostimulant application to herbicide-damaged plants is still scarce. Therefore, the current research aimed to study the effect of simulated herbicide drift from Clearfield oilseed rape field to conventional oilseed rape and to evaluate the effect of additionally applied plant biostimulant for medicative treatment to the conventional oilseed rape hybrid.

Materials and Methods

Treatments of the field trial

The field trial included the following treatments:

1. Untreated control;
2. Cleranda SC – $0.40 \text{ L} \cdot \text{ha}^{-1}$ (20 % of the rate $3.00 \text{ L} \cdot \text{ha}^{-1}$);
3. Cleranda SC – $0.60 \text{ L} \cdot \text{ha}^{-1}$ (30 % of the rate $3.00 \text{ L} \cdot \text{ha}^{-1}$);
4. Cleranda SC – $0.40 \text{ L} \cdot \text{ha}^{-1}$ + Amino Expert Impuls – $3.00 \text{ L} \cdot \text{ha}^{-1}$;
5. Cleranda SC – $0.60 \text{ L} \cdot \text{ha}^{-1}$ + Amino Expert Impuls – $3.00 \text{ L} \cdot \text{ha}^{-1}$.

Used herbicidal and biostimulant products

Herbicide: Cleranda® SC ($17.5 \text{ g} \cdot \text{L}^{-1}$ imazamox + $375 \text{ g} \cdot \text{L}^{-1}$ metazachlor) is a herbicidal product that can be applied for control of a range of dicotyledonous and monocotyledonous weeds in Clearfield winter and spring oilseed rape varieties (BASF 2025).

Biostimulant: Amino Expert Impuls (Ecofol AD 2025) (Amino acids – 5.00 %

(free amino acids – 4.43 %); Macroelements: N – 2.53 %; MgO – 0.50 %; SO_3 – 4.02 %; Phytohormones – 0.0003 %; Organic substances and natural adhesives: 73.96 %; Microelements: B – 0.52 %; Cu – 0.39 %; Fe – 0.38 %; Mn – 0.38 %; Mo – 0.08 %; Zn – 0.78 %).

Trial design

The trial was conducted using the randomised block design in three replications with an experimental plot size of 10 m^2 .

The imitation of herbicide drift was performed in crop growth stage three-four leaves unfolded (BBCH 14-15). The biostimulant Amino Expert Impuls was applied four days after the herbicidal drift. The spraying was done with backpack sprayer SOLO model 417 (Solo, Germany) with a spraying solution of $250 \text{ L} \cdot \text{ha}^{-1}$ for both treatments.

The preceding crop for the oilseed rape in the two experimental years was winter wheat. On the trial field, deep ploughing, two times disc harrowing, and two times cultivation before sowing was done. The whole experimental area was fertilised with $250 \text{ kg} \cdot \text{ha}^{-1}$ NPK 15:15:15 and $200 \text{ kg} \cdot \text{ha}^{-1}$ NH_4NO_3 as spring dressing.

The oilseed rape grown in the study was the hybrid PT225 which is one of the most cultivated conventional hybrids with Pioneer® brand in Bulgaria. The hybrid PT225 has excellent pod shattering resistance, maximum yield potential and high oil content and is suitable for late sowing as well. It also has tolerance to economically important diseases and very good cold tolerance.

Studied indicators

Symptoms of herbicidal damages (scores).

The visual scoring of the damages caused by the herbicide drift was assessed by the 9-score visual scale of European Weed Research Society (EWRS) on the 7th, 14th, and 28th days after treatment of the imitation of the herbicide drift. The scores are described below:

1. No effect (healthy plants);
2. Very slight effects; some stunting and yellowing just visible;
3. Slight effects; stunting and yellowing, effects reversible;
4. Substantial chlorosis and/or stunting, most effects probably reversible;
5. Strong chlorosis/stunting; thinning of stand;
6. Increasing severity of damage;
7. Increasing severity of damage;
8. Increasing severity of damage;
9. Total loss of plants and yield.

Number of primary branches per plant (NPB): measurements were taken at the end of the growing season on 25 plants per plot (75 plants in total per treatment).

Plant height (PH, cm): measurements were taken at the end of the growing season on 25 plants per plot (75 plants in total per treatment).

Oilseed rape seed yield (SY): it was recorded by harvesting the entire plot of each treatment. Harvesting was performed using a Wintersteiger field-plot combine (Wintersteiger Group). The data from each plot were recalculated to establish the seed yields per hectare ($t \cdot ha^{-1}$).

Weight of 1000 seeds (g): three batches of 1000 seeds for each replication per each variant of the trial were measured. The results for each replication were recalculated by dividing the results of the three batches of 1000 seeds by three to make the average sample for each plot ($3 \cdot 3 = 9$ batches of 1000 seeds per variant) as described by Tonev et al. (2018).

Physiological parameters (in 2021)

Total chlorophyll content ($mg \cdot m^{-2}$) at 7, 14 and 28 days after the simulated herbicide drift: a portable chlorophyll meter, model CCM-300 plus was used for measurements. Twenty plants per replication were measured (sixty plants total per treatment).

Seed oil content

The seed oil content (OC) (%) was determined by the Soxhlet method as described by Ivanov and Popov (1994). Three samples per treatment were analysed.

Agrometeorological data

Agrometeorological data during the experiments were obtained from the automatic meteorological station Meteobot® Pro situated by the experimental field. The data is for the average monthly minimum and maximum air temperatures ($^{\circ}C$) as well as precipitation (mm) for the growing seasons of the oilseed rape during the two years of the study (2020/2021 and 2021/2022).

Statistical analyses of the collected data

Statistical analysis of the obtained results was processed using one-way ANOVA followed by Duncan's Multiple Range Test, implemented in SPSS version 26.

Results and Discussion

Meteorological data

Figures 1 and 2 present the average monthly precipitation and the minimum

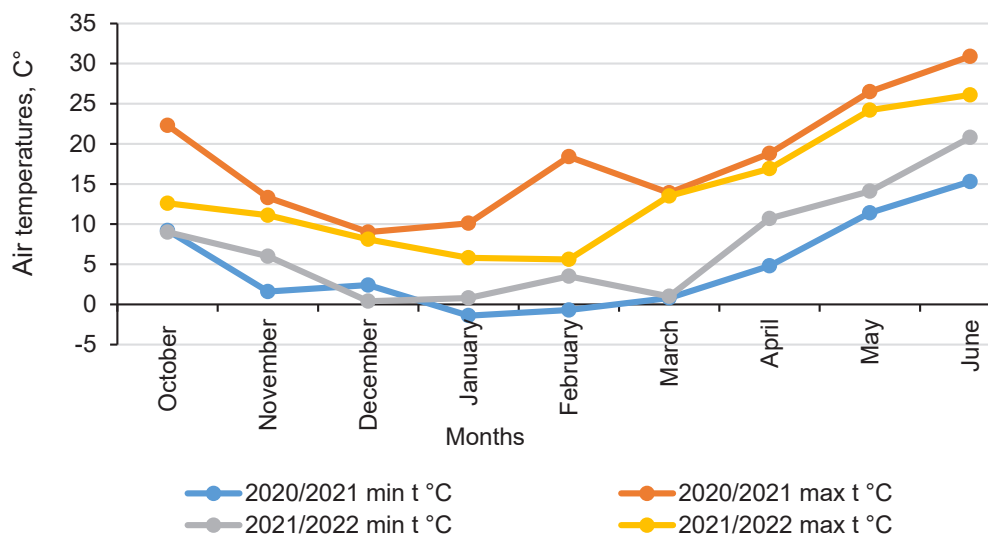


Fig. 1. Average monthly minimum and maximum air temperatures from October to June during the two experimental growing seasons.

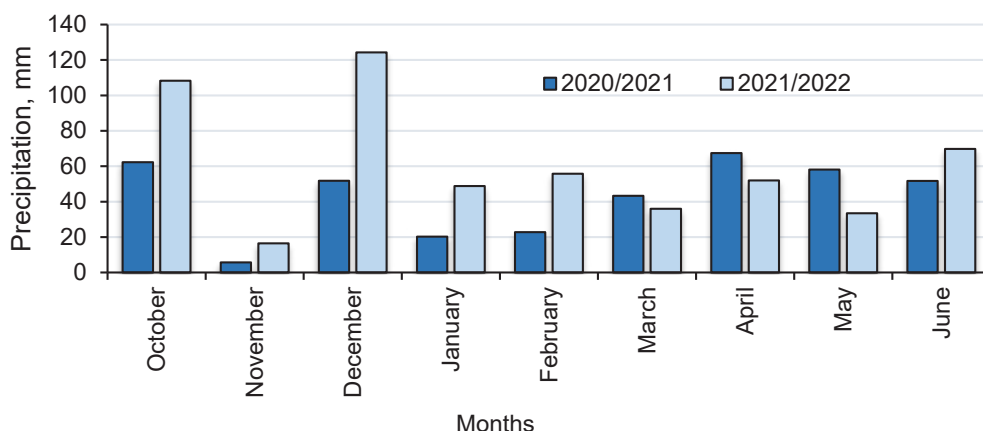


Fig. 2. Average monthly precipitation from October to June during the two experimental growing seasons.

and maximum air temperatures recorded during the oilseed rape growing seasons of 2020/2021 and 2021/2022.

According to the WMO (2010), all pesticides (herbicides, insecticides, fungicides, and rodenticides), as well as growth regulators and fertilisers, require special attention to the meteorological conditions.

In a trial performed by Tonev et al. (2011), the agroecological conditions in the period of herbicide treatment and the first days after application influenced the retention and absorption of foliar herbicides. Before or after the imitation of the herbicide drift, no temperatures that can contribute to the evaluations of the phyto-

toxic symptoms were recorded. If rain falls immediately or during the herbicide spraying, it can wash away any unabsorbed amount of the pesticide. The precipitation amount during imitation of the herbicidal drift in the two experimental years did not affect phytotoxicity and its overcoming by the oilseed rape plants (Fig. 2).

The precipitation was not equally distributed during the oilseed rape growing seasons. As summarised, the total amount of rainfall was higher for the second experimental period (2021/2022), especially in the first part of the growing season. Lower precipitation was recorded in the first research period (2020/2021). The precipitation during the two growing seasons was enough for the plant's growth and development. No water stress was observed during the investigation periods.

The average minimum and maximum air temperatures were different during the two growing seasons of the oilseed rape but suitable for the plants. No extreme values affecting the crop were recorded.

It is summarised that a negative influence of the precipitation and air temperatures was not found.

Symptoms of phytotoxicity caused by the imitation of the herbicide drift

The imazamox that belongs to the imidazolinones is a herbicide that inhibits acetolactate synthase (ALS). This is one of the herbicide-active substances in the herbicidal product Cleranda SC. The visual phytotoxic symptoms from imazamox observed on non-target crops are growth retardation, leaves turgor loss, plants losing their vitality, etc. (Fedke and Duke 2005). In the current experiment, phytotoxicity is considered to be caused by imazamox because the other ingredient is metazachlor. It is known to be selective for the oil-

seed rape (Dimitrova et al. 2014). Neshev et al. (2021b) performed a trial imitating the case of herbicide drift of herbicidal solution intended for weed management in wheat where the higher dose of the herbicide used in the study caused more severe damage to the neighbouring oilseed rape.

In the recent study, these statements were reconfirmed (Fig. 3). On the 7th day after the herbicide drift imitation the visual phytotoxic symptoms caused by the imitation of the herbicide drift were more severely expressed in the plants that received 30 % of 2.00 L·ha⁻¹ registered rate of Cleranda. The plants sprayed with 20 % equivalent of 2.00 L⁻¹·ha⁻¹ had lower phytotoxic performance. The plants that received a medicative application of the plant biostimulant Amino Expert Impuls recovered to some extent. The plants that received 20 % of Cleranda rate overcame the herbicidal stress caused by the herbicide drift. Their growth and development were improved compared to the plants not receiving biostimulant application. Similar findings from studies worldwide have confirmed the ameliorative application of different biostimulants after herbicide damages in different crops such as oats and winter wheat (Soltani et al. 2015), sunflower (Balabanova et al. 2016), pumpkins (Neshev et al. 2020, 2021a), oilseed rape (Neshev et al. 2021b), and maize (Soltani et al. 2015, Balabanova et al. 2021).

In the second assessment date (14 days after the herbicide drift) the phytotoxicity for treatments 4 Cleranda SC – 0.40 L·ha⁻¹ + Amino Expert Impuls – 3.00 L·ha⁻¹) and 5 (Cleranda SC – 0.60 L·ha⁻¹ + Amino Expert Impuls – 3.00 L·ha⁻¹) decreased to 2. The phytotoxicity at variant 3 (Cleranda SC – 0.60 L·ha⁻¹) decreased to 4, and the phytotoxicity for treatment 2 (Cleranda SC – 0.40 L·ha⁻¹) was diminished to 3.

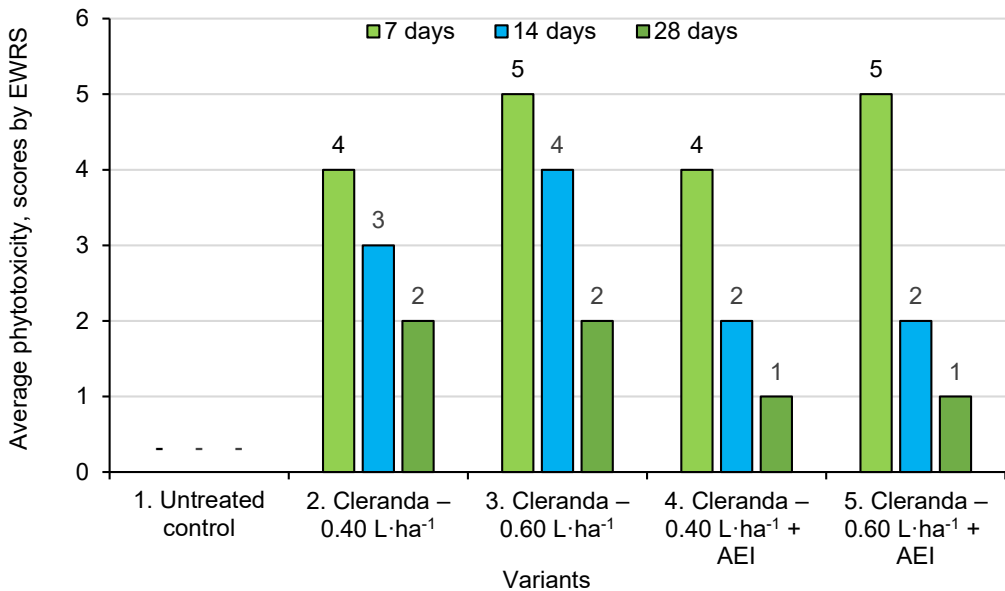


Fig. 3. Average phytotoxicity scores according to EWRS 9-point scale.

On the last evaluation date (28 days after the herbicide drift), the plants treated with Amino Expert Impuls had a phytotoxicity score of 1, which indicates that the application of the biostimulant improves the status of the plants of the experiment. In the plants without ameliorative biostimulant treatment, the phytotoxicity on the 28th day was with a score of 2, and a very weak suppression of the rape plants was still observed.

Physiological parameters: Total chlorophyll

The total chlorophyll content differed between the treatments (Table 1). Sharma et al. (2018) found that the application of pesticides can cause oxidative stress to plants. This oxidative stress can result in chlorophyll degradation that is reducing the plants' photosynthetic efficiency (Xia et al. 2006, Badr et al. 2013, Sharma et al.

Table 1. Total chlorophyll content (mg·m⁻²) in 2021.

Treatments	Days after the herbicide drift		
	7	14	28
1. Untreated control	375 a	414 a	432 a
2. Cleranda SC – 0.40 L·ha ⁻¹	212 c	259 c	318 c
3. Cleranda SC – 0.60 L·ha ⁻¹	154 d	210 d	224 d
4. Cleranda SC – 0.40 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	314 b	354 b	397 b
5. Cleranda SC – 0.60 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	194 d	218 d	246 d

Note: Means followed by different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

2015, Varshney et al. 2015).

Compared to control there was a significant decrease of the total chlorophyll content because of the herbicidal drift. When the oilseed rape plants were stressed to a greater degree (after receiving 30 % of the 2.00 L·ha⁻¹ registered rate of Cleranda) the total chlorophyll in the leaves was diminished. Higher total chlorophyll content was measured in the leaves decreased significantly compared to control. Higher total chlorophyll content was measured in the leaves of the plants that had ameliorative application of biostimulant. The data obtained correspond with results recorded by Neshev et al. (2021b), where an improvement effect of plant biostimulant regarding the plastid pigments content was found.

The results for the content of total chlorophyll in the leaves showed an increase in the chlorophyll content over time in all treatments.

On all measurement dates, the highest content of total chlorophyll in oilseed rape leaves was found in the untreated control – 375, 414 and 432 mg·m⁻² (7, 14 and 28 days after treatment, respectively).

On the 7th day after the imitation of the herbicide drift, the lowest values for the indicator were found in variant 3 – 154 mg·m⁻². Seven days after spraying in variant 2, the content of total chlorophyll was slightly higher, at 212 mg·m⁻². An increase in the chlorophyll content in the leaves was found during the subsequent measurement dates.

A positive influence of the medicinal treatment with the biostimulant Amino Expert Impulse was reported regarding the content of total chlorophyll in leaves. The positive effect was higher in the variant treated with 20 % of the herbicide Cleranda, where the content of total chlorophyll in the leaves was 314 mg·m⁻² on the 7th

day after the herbicide drift, and the increase in the result was significant.

Number of primary branches per plant and plant height

NPB is presented in Table 2. The highest number of branches per plant were counted for the untreated control – 7.65 on average across the study period. The lowest results for plants in which herbicide drift was simulated with 20 and 30 % of the rate 2.00 L·ha⁻¹ were reported – 3.85 and 3.60 branches per plant. For the treatments where a remedial biostimulant application was sprayed, the plants were found to increase the number of branches to some extent.

In research findings, the reports of Varshney et al. (2015), Neshev et al. (2020), and Marques et al. (2021) concerning the diminishing of the growth parameters of plants that were exposed to herbicidal stress are reconfirmed.

The indicators of NPB per plant and PH at the end of the growing season were lower when compared to the untreated control plants. A higher decrease was obtained for the plants that received 30 % of the 2.00 L·ha⁻¹ registered rate of Cleranda (Treatment 3). As found by other scientists, the growth traits are enhanced after the application of plant biostimulant for ameliorative treatment (Calvo et al. 2014; Constantin et al. 2016; Neshev et al. 2021b, 2022; Balabanova et al. 2023).

Oilseed rape seed yield

It was found that herbicide drift and the subsequent herbicidal stress can cause a severe yield decrease (Schroeder et al. 1983, Deeds et al. 2006, Roider et al. 2008, Webster et al. 2016, Neshev et al. 2021b, Balabanova et al. 2023).

Table 2. Number of primary branches and plant height (cm) at the end of the growing season.

Treatments	Experimental seasons		Average
	2020/2021	2021/2022	
Number of primary branches per plant			
1. Untreated control	7.20 a	8.10 a	7.65
2. Cleranda SC – 0.40 L·ha ⁻¹	4.80 b	6.30 b	5.55
3. Cleranda SC – 0.60 L·ha ⁻¹	3.60 d	4.10 c	3.85
4. Cleranda SC – 0.40 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	5.50 b	5.90 b	5.70
5. Cleranda SC – 0.60 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	4.00 c	4.40 c	4.20
Plant height, cm			
1. Untreated control	1.55 a	1.63 a	1.59
2. Cleranda SC – 0.40 L·ha ⁻¹	1.08 c	1.16 b	1.12
3. Cleranda SC – 0.60 L·ha ⁻¹	0.95 d	0.99 c	0.97
4. Cleranda SC – 0.40 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	1.18 b	1.27 b	1.23
5. Cleranda SC – 0.60 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	1.11 c	0.97 c	1.04

Note: Means followed by different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

In our study the highest SY was recorded in the untreated control (2.68 t·ha⁻¹). The treatments in which the biostimulant treatment was applied increased their yields compared to the treatments that received 20 and 30 % of 2.00 L·ha⁻¹ of Cleranda herbicide. After the imitation of the herbicidal drift the obtained seed yields for these treatments were 2.24 and

1.50 t·ha⁻¹ respectively average for both experimental years (Table 3). The seed yields for the treatments where only imitation of herbicide drift was carried out were significantly lower compared to the control – 2.02 t·ha⁻¹ for the 20 % and 1.24 t·ha⁻¹ for the 30 %.

The yields for the plants that received 20 and 30 % of the herbicidal rate

Table 3. Oilseed rape seed yield (SY), t ha⁻¹.

Treatments	Experimental seasons		Average
	2020/2021	2021/2022	
1. Untreated control	2.47 a	2.88 a	2.68
2. Cleranda SC – 0.40 L·ha ⁻¹	1.88 b	2.15 b	2.02
3. Cleranda SC – 0.60 L·ha ⁻¹	1.09 d	1.39 c	1.24
4. Cleranda SC – 0.40 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	2.11 b	2.37 b	2.24
5. Cleranda SC – 0.60 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	1.51 c	1.48 c	1.50

Note: Means followed by different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

2.00 L·ha⁻¹ of Cleranda were lower. With the application of biostimulants an increase in crop productivity was recorded (Panfili et al. 2019; Neshev et al. 2020, 2021a, 2021b, 2022; Balabanova et al. 2023). The findings correspond with the data from the recent study. The plants that received ameliorative plant biostimulant treatment showed seed yield increase in comparison to the variants where no spraying of ameliorative product was done.

Weight of 1000 seeds

According to Georgiev et al. (2014), the parameter weight of 1000 seeds is of great importance for the crops' productivity. The weight of 1000 seeds of the oilseed rape differed among the treatments as well.

The untreated control had the highest results for the indicator (Table 4). It was found that herbicidal stress can decrease the weight of 1000 seeds of different crops (Mitkov et al. 2019; Neshev et al. 2021b, 2022). The mentioned finding is confirmed in the present research as well. The plants that received inappropriate herbicidal solution by the herbicide drift had the lowest weight of 1000 seeds, and after the

medicative biostimulant application, the indicator was enhanced when compared to the plants treated with the wrong herbicide only.

As with all the investigated indicators, and with this indicator, the highest results were in the untreated control – 4.25 g average for the period. The weight of 1000 seeds of the plants treated with the plant biostimulant was 3.93 and 3.41 g, respectively, for the 20 and 30 % of the herbicidal product Cleranda. The lowest results for the indicator for the plants that received 30 % of the 2.00 L·ha⁻¹ rate of the herbicide – 3.21 g.

Seed oil content

As for all other evaluated parameters, differences between the treatments were found for the SO content (Table 5).

In the seeds of the untreated control plants, the highest SO was measured, with an average of 49.8 % for the trial period. After the analyses, the lowest SO for treatment 3 was recorded – 43.3 %. After the ameliorative biostimulant application, a slight increase in SO for treatments 4 and 5 was found – 47.0 and 43.9 % average for the research conditions.

Table 4. Weight of 1000 seeds, g.

Treatments	Experimental seasons		Average
	2020/2021	2021/2022	
1. Untreated control	4.14 a	4.34 a	4.25
2. Cleranda SC – 0.40 L·ha ⁻¹	3.64 b	3.73 b	3.69
3. Cleranda SC – 0.60 L·ha ⁻¹	3.25 c	3.17 d	3.21
4. Cleranda SC – 0.40 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	4.04 ab	3.81 b	3.93
5. Cleranda SC – 0.60 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	3.51 b	3.30 c	3.41

Note: Means followed by different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

Table 5. Seed oil content, %.

Treatments	Experimental seasons		Average
	2020/2021	2021/2022	
1. Untreated control	48.5 a	51.0 a	49.8
2. Cleranda SC – 0.40 L·ha ⁻¹	44.4 c	46.7 b	45.6
3. Cleranda SC – 0.60 L·ha ⁻¹	42.6 e	44.0 c	43.3
4. Cleranda SC – 0.40 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	46.7 b	47.3 b	47.0
5. Cleranda SC – 0.60 L·ha ⁻¹ + Amino Expert Impuls – 3.00 L·ha ⁻¹	43.3 d	44.5 c	43.9

Note: Means followed by different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

Conclusion

Herbicide drift induces phytotoxic symptoms in the conventional oilseed rape hybrid PT 225, and the damage is more severely pronounced at 30 % of the high registered rate (2.00 L·ha⁻¹) of the herbicidal product Cleranda. The plants that received a medical biostimulant treatment recover faster and show higher results for the studied parameters such as plant height, number of branches, seed yield, weight of 1000 seeds, and seed oil content. The increased values of the physiological indicator total chlorophyll in the leaves confirmed the improving effect of the application of the plant biostimulator Amino Expert Impulse.

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New data on lumbricid fauna from forests in the Western Stara Planina Mountains, Bulgaria

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Thematic field: Ecosystem biodiversity, conservation and environmental restoration

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Abstract

Between 2016 and 2018, during a study on deadwood biomass stocks and their role in forest ecosystems of the Berkovski Balkan region (Western Stara Planina Mountains, Bulgaria), we investigated earthworms (Oligochaeta: Lumbricidae) captured using pitfall traps. With the help of 156 pitfall traps set in 13 sampling plots, we collected over 500 individuals belonging to 20 species and 9 genera. There are four new records for the region of the Western Stara Planina Mts.: *Dendrobaena balcanica* (Černosvitov, 1937), *Dendrobaena depressa* (Fitzinger, 1833), *Dendrobaena hortensis* (Michaelsen, 1890) and *Dendrobaena rhodopensis* (Černosvitov, 1937); three of them are new for the whole territory of the Bulgarian part of Stara Planina Mts. as well. Along with these species, we found three Balkan endemics: *Cernosvitovia rebeli* (Rosa, 1897), *Dendrobaena balcanica*, *Dendrobaena rhodopensis* and one rare species: *Dendrobaena hortensis*. Zoogeographical analysis showed that the peregrine species dominated, which seems typical for the earthworm diversity of Bulgaria. Pitfall trapping proved to be a suitable method for assessing species composition and collecting ecological data on oligochaete communities. It also shows potential for future use in ecosystem biomonitoring. We aimed to survey lumbricid fauna in the Western Balkan forests using a non-traditional method for collection of earthworms.

Key words: deadwood, earthworms, mountain woodlands, Western Balkan Mts., zoogeography.

Introduction

The impact of earthworms can be so profound that they are considered as 'ecosystem engineers' (Le Bayon et al. 2017). Earthworms (Oligochaeta: Lumbricidae) are very important for the soil formation and the decomposition of organic matter in terrestrial habitats. With their activities, they contribute to the structuring of the soil profile, support physical, chemical and microbiological properties of the soil and improve soil fertility (Edwards 2004, Andriuzzi 2015). Their activities, such as digging of channels, significantly contribute to the increasing of surface water infiltration and soil aeration (Lionetto et al. 2012).

Studies on earthworms in Bulgaria began at the end of the 19th century with accidental studies by foreign specialists, with the first information coming from Rosa (1897), who collected material from the region of Sliven and identified the first 7 species for the country. Černosvitov (1934, 1935, 1937) continued the research on the lumbricid fauna from different parts of the country. Later, Plisko (1963) reported 20 species, 3 of which were identified for the first time. Mihailova (1964, 1965, 1966, 1968) identified 30 species, 10 of which were new for the lumbricid fauna of Bulgaria. Šapkarev (1986) and Zicsi and Csuzdi (1986) conducted separate studies of the lumbricid fauna in Bulgaria, then Duhlińska (1973, 1977, 1979, 1988, 1990) reported 32 species, of which 2 new to the country. In the 21st century, studies on the earthworm communities continued (Uzunov 2010, Stojanović et al. 2012, Szederjesi 2013). In recent years, intensive studies have been carried out by Valchovski and some foreign specialists (e.g. Szederjesi 2013, Valchovski 2016, Valchovski and Velizarova 2016, Valcho-

vski and Mısırlıoğlu 2017, Valchovski et al. 2018, Valchovski and Teofilova 2024). A detailed study of the mountain lumbricid fauna has been carried out for the region of Sarnena Sredna Gora (Zdravkova et al. 2020). Currently, the earthworm fauna of Bulgaria includes 50 species, belonging to 15 genera and 2 families (Valchovski et al. 2018).

The Bulgarian part of Western Stara Planina Mountains is relatively well studied in terms of its fauna, but in-depth studies on earthworm communities are insufficient. The mountain range as a whole is also known as the Balkan Mountains. Previously published data for this region include a total of 21 earthworm species from different locations (Černosvitov 1937, Plisko 1963, Georgiev and Beron 1962, Beron and Georgiev 1967, Duhlińska 1979, Šapkarev 1986, Stojanović et al. 2013, Szederjesi 2013, Valchovski 2016, Valchovski et al. 2018).

The aim of this study was to survey lumbricid fauna in forests in the Western Stara Planina Mountains combining and summarising previous records on the earthworms with new data, as well as to characterise their zoogeographical distribution types and to discuss species of conservation importance.

Material and Methods

The field investigations were carried during 2016–2018 using the pitfall traps method. Each year, the material was collected seasonally in three collecting periods: spring-summer, summer-autumn, and autumn-spring. Earthworms were collected with 156 pitfall traps placed in 13 sampling sites (Fig. 1, Table 1). At each site, 10–12 traps were set. We focused exclusively on old-growth forests

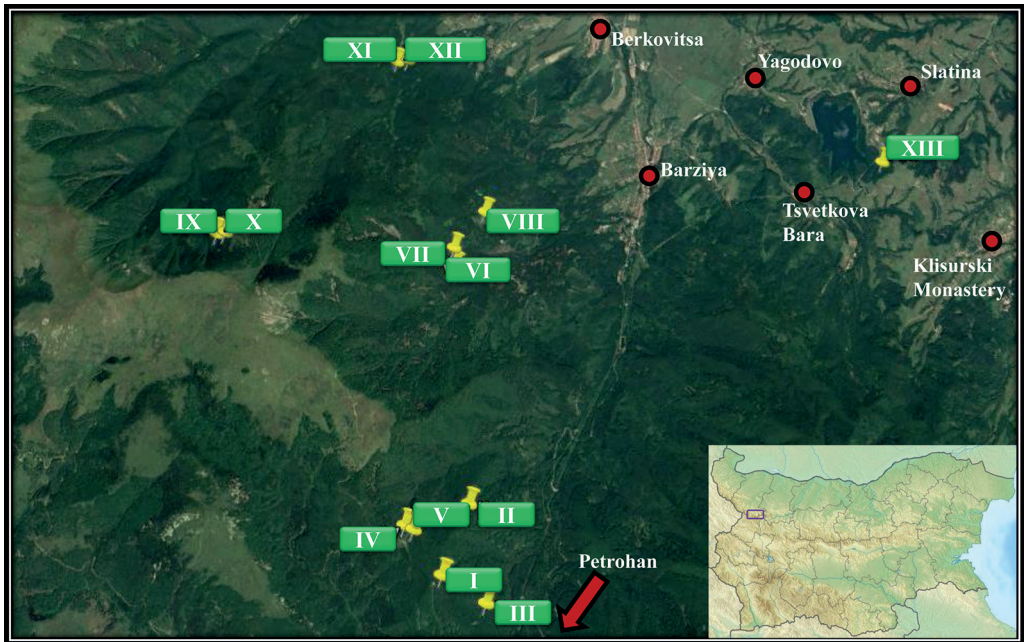


Fig. 1. Map of the locations of the sampling sites.

and searched for habitats with different amounts of very old trees and deadwood. It is worth to point out that the material was collected with a non-traditional method for collection of earthworms.

The traps were made of 2 L plastic bottles cut in the middle, buried at the level of the ground surface and filled with 300 mL of 4% formaldehyde as a fixative. They were placed in different habitats, expositions and altitudes throughout the studied region. The pitfall trap method is one of the oldest and most common ways for faunistic and ecological studies of the invertebrates such as ground beetles, spiders, ants, etc. and small terrestrial mammals. However, it has also been used experimentally and proved useful in earthworm studies as well (e.g. Zdravkova et al. 2020). Our studies on earthworms in Western Stara Planina Mts. can be divided into two periods: the first – 2016 and

2017, and the second – 2018. Some of the data on the material collected during the first period were published by Valchovski et al. (2018), but they concern material collected during the present study and are therefore discussed here as well. The material published in Valchovski et al. (2018) represents only a part of all collected specimens. Significant species were discovered during the second period as well, which is why we decided to publish them and combine the two periods to analyse the whole study. The information obtained from the first period is the reason to conduct a scientific experiment in the second period.

For the identification of the collected material, keys and tables by various authors for other territories (Chekanovskaia 1960, Perel 1979, Mršić 1991, Csuzdi and Zicsi 2003, Reynolds 2018) were used. Some of the main diagnostic characteris-

Table 1. Sampling sites in the Western Stara Planina Mts., with their average altitude, GPS coordinates, and habitat descriptions.

No	Latitude/ Longitude	Altitude, m a.s.l.	Locality and habitat
I	43°07'57" N, 23°07'10" E	1580–1630	N Petrohan Pass. Old beech forest with deadwood. Without anthropogenic impact.
II	43°08'33" N, 23°07'27" E	1390–1420	N Petrohan Pass. Clearing (100 % of the trees have been cut down), 30 % of the deadwood have not been extracted.
III	43°07'44" N, 23°07'39" E	1420–1440	N Petrohan Pass. Old beech forest with completely extracted deadwood.
IV	43°08'19" N, 23°06'49" E	1520–1540	NW Petrohan Pass. Young beech coppice. The deadwood is formed by the left branches after the felling.
V	43°08'22" N, 23°06'42" E	1520–1540	NW Petrohan Pass. Ridge meadow with juniper bushes near the ecotone bordering young beech forest. No deadwood present.
VI	43°10'56" N, 23°07'08" E	about 930	SW Barziya village. Old beech forest with dense canopy but with small amount of deadwood.
VII	43°10'45" N, 23°07'09" E	920–940	SW Barziya village. Young beech forest formed after the final phase of logging; starting succession.
VIII	43°11'18" N, 23°07'32" E	850–875	SW Barziya village. Windthrow at the place of an old beech forest. Primary succession after removal of all deadwood.
IX	43°10'55" N, 23°04'25" E	1630–1700	SW Berkovitsa. Gorna Koriya Reserve. Centuries-old spruce forest without anthropogenic impact; preserved deadwood.
X	43°10'55" N, 23°04'17" E	1580–1650	SW Berkovitsa. Gorna Koriya Reserve. Beech forest without anthropogenic impact; all deadwood is preserved.
XI	43°12'59" N, 23°06'25" E	670–720	SW Berkovitsa. Mixed deciduous forest with some trees uprooted by snow; all deadwood is preserved.
XII	43°12'59" N, 23°06'16" E	620–670	SW Berkovitsa. Mixed deciduous forest and pasture near Berkovska River with partly preserved deadwood.
XIII	43°11'55" N, 23°12'47" E	about 525	SE Berkovitsa, NE Klisurski Monastery. Old oak forest (<i>Quercus petraea</i> (Matt.) Liebl., <i>Q. robur</i> L.).

tics are the prostomium type, body length, arrangement of setae, position of clitellum and tubercula pubertatis, etc.

Following the distribution types given by Omodeo and Rota (1999), and Csuzdi et al. (2011), this analysis of the earthworm taxa recorded in the entire territory of Western Balkan Mt. has identified different distribution ranges: Peregrine, Trans-Aegean, Balkan endemic, Holomediterranean, Balkanic-Alpine, Central European, Western and Central European and Central European.

Results and Discussion

During the investigation, the species composition of the lumbricid fauna in the region was revealed. Subsequently, its diversity (rare, mass, endemic species), taxonomic and zoogeographic structure were analysed.

During the entire three-year study period, we collected over 500 specimens with a total of 20 species from 9 genera of the family Lumbricidae. This represents 40 % of all established for Bulgarian lumbricid

species and 60 % of the genera. Similarly, an extremely rich lumbricid fauna has been found in other Bulgarian mountains, such as the Sarnena Sredna Gora Mts. (21 species, 42 % of Bulgarian lumbricids) (Zdravkova et al. 2020) and the Western Rhodopes (23 species, almost 50 %) (Valchovski and Teofilova 2024), as well.

During the first stage of the work (2016–2017), around 150 specimens were determined and the data were included in the general faunistic analysis. During the second stage (2018), 17 species from 6 genera were identified and the data from this period were used for quantitative analyses. A total of 264 specimens were identified to species level, representing 74 % of the total number of specimens. The remaining 26 % (91 specimens) were identified only to genus. This material includes torn, dried and crushed individuals. Of the preserved material, 63 % are sexually mature individuals, and the rest are juveniles.

The annotated species list below includes all species occurring in the Western Stara Planina Mts. (SPW), encompassing both those identified in our study and species known only from the literature, without new material. Species marked with an asterisks (*) represent part of the material from the first period of our survey; they were collected in 2016–2017 and were already published by Valchovski et al. (2018).

Species list

1. *Allolobophora chlorotica* (Savigny, 1826)

Distribution: Peregrine. **Distribution in SPW:** Slivnica (Šapkarev 1986)

Habitat: under rocks on river bank (Plisko 1963); meadow and forest (Stojanović et al. 2012)

Material examined: N/A

2. *Aporrectodea caliginosa* (Savigny, 1826)

Distribution: Peregrine. **Distribution in SPW:** Svoге – Iskar River (Plisko 1963)

Habitat: on river banks (Plisko 1963); oak forest (Stojanović et al. 2012)

Material examined: N/A

3. *Aporrectodea handlirschi* (Rosa, 1897)

Distribution: Trans-Aegean. **Distribution in SPW:** Slivnica (Šapkarev 1986)

Habitat: in oak forest, pasture and river bank (Zdravkova et al. 2020)

Material examined: N/A

4. *Aporrectodea jassyensis* (Michaelsen, 1891)

Distribution: Trans-Aegean. **Distribution in SPW:** Dragoman (Šapkarev 1986)

Habitat: meadows and cultivated soils (Mihailova 1966); forests (Szederjesi 2013); oak forest (Stojanović et al. 2012)

Material examined: N/A

5. *Aporrectodea rosea* (Savigny, 1826)

Distribution: Peregrine. **Distribution in SPW:** Todorova Dupka Cave, Vratsa (Plisko 1963), Dragoman (Šapkarev 1986)

Habitat: in caves (Plisko 1963); meadow and forests (Stojanović et al. 2012)

Material examined: site III: 1 ex.; site X: 1 ex.

6. *Aporrectodea trapezoides* (Dugès, 1828)

Distribution: Peregrine. **Distribution in SPW:** Dragoman (Šapkarev 1986)

Habitat: cultivated soils, river banks (Šapkarev 1986); meadow and forest (Stojanović et al. 2012); forests (Valchovski 2014)

Material examined: N/A

7. **Bimastos eiseni* (Levinsen, 1884)

Distribution: Peregrine. **Distribution in SPW:** Gorna Koriya Reserve, Berskovska River, Barzia, Petrohan (Valchovski et al. 2018)

Habitat: living on tree bark

Material examined: site I: 5 ex.; site II: 3 ex.; site X: 1 ex.

8. **Bimastos rubidus* (Savigny, 1826)

Distribution: Peregrine. **Distribution in SPW:** Magurata Cave, Belogradchik and Svetata voda Cave, Ginci (Plisko 1963), Ginci, Ledenika Cave (Beron and Georgiev 1967), Beledie Han (Duhlińska 1979), Dragoman (Šapkarev 1986), Svoge (Valchovski 2016), Gorna Koriya Reserve, Petrohan Pass, Srechenska Bara, Berkovitsa (Valchovski et al. 2018)

Habitat: in caves (Cernosvitov 1937, Plisko 1963)

Material examined: site I: 10 ex.; site II: 4 ex.; site IV: 3 ex.

9. *Cernosvitovia biserialis* (Černosvitov, 1937)

Distribution: Balkan endemic. **Distribution in SPW:** In a cave near Vratsa (Cernosvitov 1937), Dragoman (Šapkarev 1986)

Habitat: in caves (Cernosvitov 1937)

Material examined: N/A

10. **Cernosvitovia rebeli* (Rosa, 1897)

Distribution: Balkan endemic. **Distribution in SPW:** Lukovo, Svoge (Valchovski 2016), Petrohan (Valchovski et al. 2018)

Habitat: beech forest (Valchovski 2016)

Material examined: site XIII: 2 ex.

11. **Dendrobaena misirlioglui* Csuzdi & Szederjesi, 2023

Distribution: Balkan-Alpine. **Distribution in SPW:** Gorna Koriya Reserve (Valchovski et al. 2018)

Habitat: rich in rotting organics (leaf litter and deadwood) forests (Zdravkova et al. 2020)

Material examined: site III: 1 ex.; site XII: 2 ex.

12. **Dendrobaena attemsi* (Michaelsen, 1902)

Distribution: Trans-Aegean (Misirlioglu and Stojanović 2020). **Distribution in SPW:** Gorna Koriya Reserve, Petrohan, Srechenska Bara, Berkovitsa, Barzia (Valchovski et al. 2018)

Habitat: We found it in many of the sampling sites, thus we could claim it is a forest dweller.

Material examined: site I: 4 ex.; site II: 1 ex.; site IV: 1 ex.; site V: 10 ex.; site VI: 2 ex.; site VII: 2 ex.; site VIII: 1 ex.; site IX: 2 ex.; site X: 7 ex.; site XII: 1 ex.

13. *Dendrobaena balcanica* (Černosvitov, 1937)

Distribution: Balkan endemic. **Distribution in SPW:** This is the first record here

Habitat: We found it in ridge meadow with juniper and young beech forest

Material examined: site V: 2 ex.

14. *Dendrobaena byblica* (Rosa, 1893)

Distribution: Holomediterranean. **Distribution in SPW:** Ledenika Cave (Duhlińska 1979)

Habitat: oak forest (Stojanović et al. 2012)

Material examined: site XI: 4 ex.; site XII: 1 ex.

15. *Dendrobaena depressa* (Fitzinger, 1833)

Distribution: Central European. **Distribution in SPW:** This is the first record here

Habitat: We found it in mixed deciduous forest

Material examined: site XI: 1 ex.

16. *Dendrobaena hortensis* (Michaelsen, 1890)

Distribution: Western and Central European. **Distribution in SPW:** This is the first record of this species here

Habitat: near river banks (Mihailova 1966), mixed forest (Zdravkova et al. 2020)

Material examined: site V: 1 ex.

17. **Dendrobaena octaedra* (Savigny, 1826)

Distribution: Peregrine. **Distribution in SPW:** Lakatnik (Plisko 1963), Gorna Koriya Reserve, Berkovska River, Petrohan (Valchovski et al. 2018)

Habitat: under rocks (Plisko 1963); forests (Stojanović et al. 2012)

Material examined: site II: 2 ex.; site III: 4 ex.; site V: 1 ex.; site VII: 1 ex.; site XII: 1 ex.

18. **Dendrobaena platyura* (Fitzinger, 1833)

Distribution: Central European. **Distribution in SPW:** Barziya, Gorna Koriya Reserve, Berkovska River, Petrohan Pass, Berkovitsa (Valchovski et al. 2018)

Habitat: We found it in clearing, old beech, young coppice beech forest, windthrow in old beech forest, mixed deciduous forest, pasture and river bank

Material examined: site II: 3 ex.; site VI: 1 ex.; site VII: 1 ex.; site VIII: 8 ex.; site XI: 4 ex.; site XII: 7 ex.

19. *Dendrobaena rhodopensis* (Černosvitov, 1937)

Distribution: Balkan endemic. **Distribution in SPW:** This is the first record here

Habitat: We found it in beech forests

Material examined: site II: 2 ex.; site X: 1 ex.

20. *Eisenia fetida* (Savigny, 1826)

Distribution: Peregrine. **Distribution in SPW:** Slivnica (Šapkarev 1986)

Habitat: meadow and forest (Stojanović et al. 2012); rich in organic matter places (Zdravkova et al. 2020)

Material examined: site III: 1 ex.

21. **Eisenia lucens* (Waga, 1857)

Distribution: Central European. **Distribution in SPW:** Magurata Cave (Plisko 1963), Lakatnik (Duhlińska 1979), Montana (Szederjesi 2013), Berkovitsa, Gorna Koriya Reserve, Petrohan (Valchovski et

al. 2018)

Habitat: in caves (Plisko 1963), near road (Szederjesi 2013)

Material examined: site VI: 2 ex.; site XI: 3 ex.

22. **Eiseniella tetraedra* (Savigny, 1826)

Distribution: Peregrine. **Distribution in SPW:** Lakatnik (Plisko 1963), Dragoman (Šapkarev 1986), Berkovitsa (Valchovski et al. 2018)

Habitat: near river (Plisko 1963); oak forest (Stojanović et al. 2012)

Material examined: N/A

23. **Lumbricus rubellus* Hoffmeister, 1843

Distribution: Peregrine. **Distribution in SPW:** Lakatnik (Černosvitov 1937, Plisko 1963), Beledie Han (Duhlińska 1979), Dragoman, Slivnica (Šapkarev 1986), Berkovska River, Petrohan Pass, Barzia, Berkovitsa (Valchovski et al. 2018)

Habitat: under rocks (Plisko 1963); meadow and forest (Stojanović et al. 2012)

Material examined: site VII: 1 ex.; site XII: 1 ex.

24. **Lumbricus terrestris* Linnaeus, 1758

Distribution: Peregrine. **Distribution in SPW:** Slivnica (Šapkarev 1986), Srečenska Bara (Valchovski et al. 2018)

Habitats: meadow (Stojanović et al. 2012)

Material examined: site XII: 1 ex.; site XIII: 12 ex.

25. **Octodrilus transpadanus* (Rosa, 1884)

Distribution: Trans-Aegean. **Distribution in SPW:** Berkovska River (Valchovski et al. 2018)

Habitat: mixed deciduous forest, pasture and river bank (Valchovski et al. 2018)

Material examined: N/A

26. **Octolasion lacteum* (Örley, 1881)

Distribution: Peregrine. **Distribution**

in **SPW**: Lakatnik (Cernosvitov 1937), Temnata Dupka Cave (Georgiev and Beron 1962), Caves near Ginci village. (Plisko 1963), Slivnica (Šapkarev 1986), Berkovitsa, Barzia (Valchovski et al. 2018)

Habitat: near river (Černosvitov 1937); in caves (Plisko 1963); meadow and forests (Stojanović et al. 2012)

Material examined: N/A

27. *Proctodrilus tuberculatus* (Černosvitov, 1935)

Distribution: Trans-Aegean. **Distribution in SPW**: Svoge (Plisko 1963), Dragoman (Šapkarev 1986)

Habitat: on the river bank (Plisko 1963)

Material examined: N/A

Taxonomic structure – genera and species

The species composition of earthworms for the Western Stara Planina, known to date, comprises 27 species from 11 genera. Of them, 20 species were collected during the present study, and 7 were registered only from the literature and were not discovered in our study: *Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea handlirschi*, *Aporrectodea jassyensis*, *Aporrectodea trapezoides*, *Cernosvitovia biserialis* and *Proctodrilus tuberculatus*.

The genus *Dendrobaena* Eisen, 1873, which includes nine species, is the dominant faunal component, not only in our study but also in the earthworm fauna of Bulgaria (Stojanović et al. 2012). The quantitative data from 2018 showed that *Dendrobaena* species are also the most abundant (52 % of the total material). These are inhabiting mountain forest habitats and their large number in our material could be explained by the fact that the sampling sites were mainly located in

beech, spruce and mixed forests. The relatively high presence of species from the genus *Bimastos* Moore, 1893 (7.4 %) and the genus *Eisenia* Malm, 1877 (5.4 %) could be explained by the fact that they are epigeal (epigeic) species inhabiting the surface soil layer, in which a large amount of dead organic matter is present, and such habitats in the region are the forest ecosystems we studied. Other species with high presence are those of the genus *Lumbricus* Linnaeus, 1758, with 7.9 %. The species of the genus *Aporrectodea* Orley, 1885 (1.1 %) have a low presence, as these are species inhabiting flat, low-land areas, and our sampling sites were mountain habitats with an altitude from 525 m up to 1650 m. The species of the genus *Cernosvitovia* Omodeo, 1956 had the lowest occurrence (0.6 %).

The quantitative analysis of the separate species showed that the most abundant species were: *D. attemsi* (25 specimens, 7 % of all), *D. platyura* (24, 6.8 %), *B. rubidus* (17, 4.8 %) and *L. terrestris* (13, 3.7 %). With 9 specimens (2.5 %) each were *B. eiseni* and *D. octaedra*; with 5 (1.4 %) each were *E. lucens* and *D. byblica*; with 3 (0.8 %) each were *D. misirlioglu* and *D. rhodopensis*; with 2 (0.6 %) each were *Ap. rosea*, *C. rebeli*, *D. balcanica* and *L. rubellus*. The least represented (one specimen) were *D. depressa*, *D. hortensis* and *E. fetida*. The species *D. balcanica*, *D. depressa*, *D. hortensis* and *D. rhodopensis* were less abundant and have a limited distribution not only in the studied region, but apparently also on the territory of Bulgaria. Some of the less common species are of conservation importance and the small number of individuals caught proves how rare they are.

We can compare our study design and results with data from the Sarnena Gora region (Zdravkova et al. 2020), where a

total of 381 specimens and 17 species of earthworms were identified, and the most common species were *L. rubellus* (43 %), *D. misirlioglui* (31 %), *E. fetida* (27 %) and *C. rebeli* (19 %). Several rare species of conservation importance were also recorded, such as *Panoniona leoni* Michaelsen, 1891 (Navarro et al. 2023) and *D. hortensis*, together with endemic species (*D. balcanica* and *C. rebeli*). In both studies, a large amount of biological material was collected in different periods of the year and from various habitat types. With the obtained data, various ecological analyses for the group of earthworms and their potential as bioindicators of environmental quality and forest ecosystems could be carried out in the future.

Faunistic analysis – new, rare, endemic, and specific lumbricids/taxa

During our research, we identified some species of conservation and scientific importance: one species new to the fauna of Bulgaria; four new unpublished species for the Bulgarian part of the Western Stara Planina Mts. (*D. balcanica*, *D. depressa*, *D. hortensis*, *D. rhodopensis*) and three of them for the entire territory of the mountain, three Balkan endemics, and one rare species (Table 2). Some of the data have

already been published by Valchovski et al. (2018), but they concern material collected during the present investigation and are therefore also considered here. *Dendrobaena hortensis* has been reported for the Serbian part of the mountain (Stojanović et al. 2013), so the fact that we have found it is not very surprising but it is still a new geographic record for the region of study.

Dendrobaena platyura is a species new to the territory of the Western Stara Planina Mts., as well as for Bulgaria. It was published by Valchovski et al. (2018) based on specimens collected by us in the period 2016–2017. Quantitative data from 2018 showed that it is among the most abundant and common species for the study region.

The other new species for the region is the Balkan endemic *Cernosvitovia rebeli*, found at only two sampling sites, with a small number of individuals, which probably confirms the hypothesis of its relative rarity. Previously published localities of *C. rebeli* in the Western Balkan include the village of Lukovo (Svoge municipality) (Valchovski 2016) and Petrohan Pass (Valchovski et al. 2018). In Bulgaria, it is widespread, and is also found in the other two parts of the Balkan Mts. – Botev Peak, Ribaritsa, Vasiliovo (Cen-

Table 2. Species of geographical and conservation importance captured for the entire study period (2016–2018).

Species	BG new	SPW new	SP new	BALe	Rare
<i>Cernosvitovia rebeli</i>				+	
<i>Dendrobaena balcanica</i>		+	+	+	
<i>Dendrobaena depressa</i>		+	+		
<i>Dendrobaena hortensis</i>		+	+		+
<i>Dendrobaena platyura</i>	+	+	+		
<i>Dendrobaena rhodopensis</i>		+		+	

Note: BG – Bulgaria, SPW – Western Stara Planina Mts., SP – whole Stara Planina Mts., BALe – Balkan endemic.

tral) (Černosvitov 1934, Duhlińska 1979, Valchovski and Mısırlıoğlu 2017) and Sliven, Stidovska Planina Mt. (Eastern) (Rosa 1897, Valchovski et al. 2018). The new localities of the species from our investigation are at 525 m a.s.l. (site XIII). Recent molecular studies have confirmed the distinct phylogenetic status of *Cernosvitovia* and provided an emended genus diagnosis, further supporting the systematic placement of *C. rebeli* (Popović et al. 2022).

Dendrobaena balcanica is an endemic species, also new to the Western Stara Planina Mts., but also new to the entire territory of the Stara Planina Mountains. It was previously reported in Bulgaria from localities in Slavianka Mts. (Černosvitov 1937), Pirin Mts. (Zicsi and Csuzdi 1986), and Sarnena Sredna Gora Mts. (Zdravkova et al. 2020). During our study, it was recorded at 1526 m a.s.l. (site V).

The third endemic species, *D. rhodopensis*, is new to the Western Stara Planina Mts., but has also been found in the Central Stara Planina Mts. (Tetevenska Planina) (Plisko 1963), in different localities in Rila Mts. (Plisko 1963; Šapkarev 1986, Zicsi and Csuzdi 1986, Stojanović et al. 2012, Valchovski et al. 2018), Vitosha Mts. (Plisko 1963, Delchev et al. 1998) and Kostenets (Rila-Rhodopes Mts.) (Černosvitov 1937). According to Stojanović et al. (2020) *D. rhodopensis* is a strictly montane species, restricted to forest soils at high altitudes, with individuals found only between 1098 and 2518 m a.s.l. We can also confirm this, as we collected the species at 1405 m a.s.l. at site II and at 1625 m a.s.l. in Gorna Koriya Reserve, at the upper limits of the beech forests (site X).

The rare species *D. hortensis* was recorded for the first time in the studied area. In Bulgaria, the species has previously

been recorded at Sarnena Sredna Gora Mts. (Mihailova 1966; Zdravkova et al. 2020) and the Strandzha Mts. (Szederjesi 2013). During our survey, it was collected at 1526 m a.s.l. (site V).

Zoogeographic characteristics of the lubricofauna

The analysis of our results obtained during this study and the data already known for the region showed that according to the zoogeographical distribution types, the earthworm fauna of the Western Balkan Mts. is dominated by peregrine species (12 taxa, 44.4 %) (*B. rubidus*, *L. rubellus*, *D. octaedra* and others), followed by Trans-Aegean (5 taxa, 18.5 %) (*A. handlirschi*, *A. jassyensis*, *D. attemsi*, *O. transpadanus*, *P. tuberculatus*), Balkan endemic (4 taxa, 14.8 %) (*C. biserialis*, *C. rebeli*, *D. balcanica*, *D. rhodopensis*), Central-European (3 taxa, 11.1 %) (*D. depressa*, *D. platyura*, *E. lucens*). Less numerous are the Balkanic-Alpine, Holo-mediterranean and Western and Central European categories with only one species each (3.7 %) (*D. misirlioglu*, *D. bybli*ca and *D. hortensis*, respectively). Similarly, peregrine species predominated in the Western Rhodopes (43.5 %), and were followed by the endemic species (21.7 %) (Valchovski and Teofilova 2024), as well. Such ratio is typical of the earthworm diversity of Bulgaria (Valchovski 2012, Stojanović et al. 2012). However, the degree of endemism is lower than the average for Bulgaria (Stojanović et al. 2012).

Quantitative representation of the species and individuals

We analysed the quantitative representation of lumbricid species at each sampling site and their dependence on the amount

of deadwood, based on the material collected in 2018. Some of the sites with a large amount of deadwood biomass and relatively large species diversity of earthworms were sites II (clearing) and VIII (windthrow with abandoned poor-quality deadwood). Other places with high quantitative activity were site XII (near a river), XI (mixed forest), and XIII (oak forest). The reason for such activity of earthworms in these places is that they are all characterised by a large amount of deadwood, a constant amount of moisture and diverse food base. Sampling sites poor in species and quantitative diversity were those with a limited amount of deadwood or the food supply had already been fully utilised (sites IV, VI and IX). These regularities showed the earthworms' activity at individual sites and confirmed their dependence on the food supply (see Edwards 2004).

The scientific experiment carried out to analyse biological material from earthworms collected with pitfall traps showed the suitability of the method for establishing the species composition and some qualitative and quantitative characteristics of lumbricid faunas. The method seems also suitable and promising for ecosystem biomonitoring, but experimental calibration and refinement of the system of biotic parameters used is necessary (see Zdravkova et al. 2020). Among the advantages of the method are its year-round duration, relatively low cost and the possibility to use it simultaneously with the collection of biological material and information on other epigeobionts and pedobionts. In practice, pitfall traps represent an independent sample or collection, and its exposure is constant. The method has some disadvantages, it is not quite proper for endogeic earthworm species, which inhabit permanently the humus soil layer. Nevertheless, the pitfall trapping is very

useful method for epigeic and most of the anecic species.

Conclusions

The lumbricid fauna of the Western Stara Planina Mts. is extremely rich and represents 40 % of all established lumbricid species recorded in Bulgarian. This is probably due to the location of the studied areas, rather humid and stable habitats, the presence of rich food base and also the sampling efforts. Here we also report 5 new species for the territory of Western Stara Planina and some species with conservation and scientific significance (three Balkan endemic species and one rare species).

Faunistic analyses obtained from the experiment using pitfall traps proved the advantages of this method over traditional ones, which are narrowly limited in space and time. The identification of new species with this non-traditional method showed the need for future studies of the earthworm fauna to apply methods with a prolonged or frequent sampling period and a relatively large number of samples, which would guarantee the capture of rare, regionally specific and stenobiont species.

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New chorological data for the flora of Sakar Mountain, South-eastern Bulgaria

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Thematic field: Ecosystem biodiversity, conservation and environmental restoration

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Abstract

The flora of Sakar Mt. is far from well-studied. The territory of the mountain is shared between two floristic regions in Bulgaria – Tundzha Hilly Country and Thracian Lowland. The aim of the study is to improve the knowledge of the floristic composition of these regions. We exploited standard methods widely used in floristic research. Resulting from the study we report eight vascular plant species from Sakar Mt. as new for the Tundzha Hilly Country floristic region incl. *Pulsatilla montana* (Hoppe) Rchb. and *Osyris alba* L., and three species as new for the Thracian Lowland floristic region incl. *Legousia pentagonia* (L.) Thell. assessed as Vulnerable in the Red List of Bulgarian vascular plants. The distribution of four species in the Tundzha Hilly Country floristic region is confirmed. The presence in Sakar Mt. of an interesting member of *Campanula rotundifolia* group is briefly discussed.

Key words: chorology, new floristic records, phytogeography, vascular plants.

Introduction

Sakar Mountain is a low mountain and hilly area located in Southeast Bulgaria and Northwest Turkey, with elevation between 50 and 856 m at the highest peak *Vishegrad* (Kolev *et al.* 2002). *It hosts very rich biodiversity in various biotope types* – grasslands, diverse types of natural forests and plantations (*Quercus cerris* L., *Quercus frainetto* Ten., *Pinus nigra* J.F. Arnold, etc.), rock formations (schists, limestone, etc.), freshwater ponds and arable land. The climate of the area is transitional continental with certain Mediterranean influence (Assenov 2021). The territory of the mountain is shared between two floristic regions in Bulgaria – Tundzha Hilly Country and Thracian Lowland.

The territory of Sakar Mountain is of significant interest from a botanical point of view, given its transitional biogeographical position and the increasing Mediterranean influence in the context of global climate change.

The existing information in scientific publications about the flora of the region is outdated or partial. First data on plant species distributed in the territory of Sakar can be found in the works of Velenovský (1891, 1898) and Podpéra (1902). The main publication on the subject is the study of Jordanov (1944) listing 420 species for Sakar Mountain distributed by vegetation types. Later, only individual floristic contributions were published (Stoyanov and Savchev 1965, Jordanov and Yanev 1968, Delipavlov and Cheshmedzhiev 1984, Assyov and Vassilev 2004, Vladimirov *et al.* 2012, Bancheva and Delcheva 2016, Petrova and Vassilev 2016, Stoyanov 2016, Stoyanov and Marinov 2016, Marinov and Stoyanov 2019, Stoyanov and Marinov 2021). Apart from the purely floristic studies the inventory of the Bulgarian flora

produced some new for the science species which to date remain local endemic for Sakar Mt. (Bancheva and Raimondo 2013). Nevertheless, the region remains relatively rarely visited and poorly known. One possible explanation for this fact is the access restriction to Sakar which existed in the past as a border area.

Considering that the main publications on the flora of Sakar Mountain are relatively old, and that taxonomic concepts have changed rapidly in recent decades, a new comprehensive study of the area is necessary.

The flora of Sakar has piqued our interest in recent years, and we have conducted numerous field trips in the area, resulting in interesting findings. The purpose of this research is to contribute to the knowledge of the plant diversity of Sakar Mountain.

Materials and Methods

The transect method was used for the study. In the period 2016–2025 more than 20 field visits have been conducted, covering the mountain territory as evenly as possible and including a variety of habitats. Three Bulgarian herbaria (SO, SOM and BNHM) have been consulted and the reported here species have been checked for localities in the area. The identification was achieved with the use of Flora of Bulgaria (Vol. 1–11), Flora Europaea (Vol. 1–5) and determination keys (Andreev *et al.* 1992, Delipavlov *et al.* 2003, Stoyanov *et al.* 2022). The nomenclature follows the Euro+Med PlantBase. The names of taxa given in parentheses are according to the Conspectus of Bulgarian Vascular Flora (Assyov *et al.* 2012). The boundaries of the floristic regions are defined according to Jordanov (1966). The names and

conservation categories of the protected areas are according to the Register of protected areas in Bulgaria (2025). The localities of the reported taxa are shown in Figure 1.

Results and Discussion

Species new for the Tundzha Hilly Country floristic region

Aconogonon alpinum (All.) Schur (syn. *Pleuropterypyrum undulatum* A. Löve & D. Löve) (Polygonaceae)

Specimen examined: Sakar Mt., Dervishka Mogila peak, between rocks, 670 m a.s.l., 41°54'49.4" N and 26°21'01.4" E, 30.05.2020, with flowers and fruits, coll.

A. Petrova and R. Vassilev (BG-NMNH-BOT-5496).

So far, this species has been known only from mountain areas above 1500 m in the floristic regions of Stara planina (Western, Central), Vitosha, Rila, Pirin (Northern), West Frontier Mountains and Belasitsa (Asenov 1966, Assyov et al. 2012, Stoyanov et al. 2022). Its finding in Sakar Mountain at relatively low altitude significantly extends the distribution range of the species in Bulgaria. The locality is part of the protected area Skalnite Obrazuvania v Mestnost Dervishka Mogila nature monument (Register of protected areas in Bulgaria 2025).

Aethionema saxatile (L.) W. T. Aiton (Brassicaceae)

Map Legend

- Sakar Mt. boundaries
- Floristic regions border

Species localities:

1. *Aconogonon alpinum*
2. *Aethionema saxatile*
3. *Aristolochia pallida*
4. *Geranium macrorrhizum*
5. *Hippocrepis emerus*
6. *Mercurialis perennis*
7. *Osyris alba*
8. *Pulsatilla montana*
9. *Acer monspessulanum*
10. *Digitalis grandiflora*
11. *Tephrosieris papposa*
12. *Vitis vinifera*
13. *Asplenium adiantum-nigrum* subsp. *onopteris*
14. *Legousia pentagonia*
15. *Paragymnopteris marantae*
16. *Campanula rotundifolia* gr.

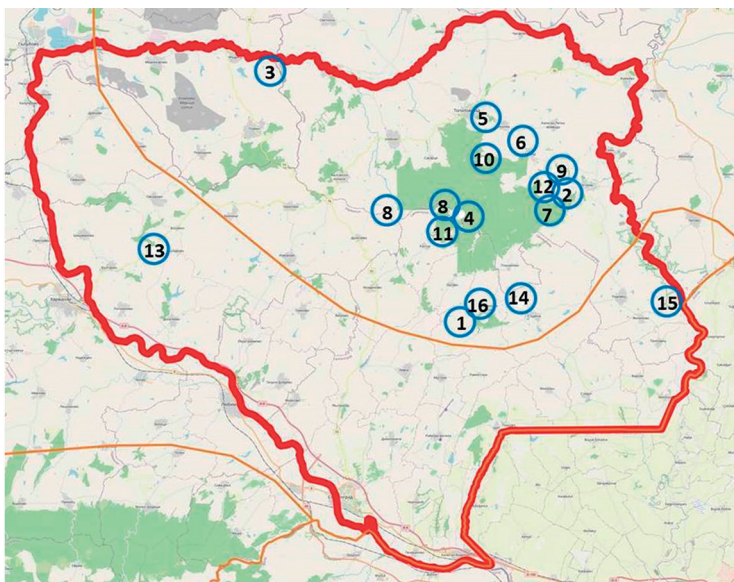


Fig. 1. Location of reported taxa.

Note: 1. *Aconogonon alpinum* (All.) Schur; 2. *Aethionema saxatile* (L.) W. T. Aiton; 3. *Aristolochia pallida* Willd.; 4. *Geranium macrorrhizum* L.; 5. *Hippocrepis emerus* (L.) Lassen; 6. *Mercurialis perennis* L.; 7. *Osyris alba* L.; 8. *Pulsatilla montana* (Hoppe) Rchb.; 9. *Acer monspessulanum* L.; 10. *Digitalis grandiflora* Mill.; 11. *Tephrosieris papposa* (Rchb.) Schur; 12. *Vitis vinifera* L.; 13. *Asplenium adiantum-nigrum* subsp. *onopteris* (L.) Heufl.; 14. *Legousia pentagonia* (L.) Thell.; 15. *Paragymnopteris marantae* (L.) K. H. Shing; 16. *Campanula rotundifolia* gr.

Specimen examined: Sakar Mt., along dry grassy areas between Bulgarska Polyana and Hlyabovo villages, 13.05.2002, coll. D. Stoyanov (SO 102256); Sakar Mt., State Forest of Sveta Troitsa Monastery nature monument, Topolovgrad Municipality, on calcareous rocky slope, 120 m a.s.l., 42°01'27.7" N and 26°26'11.1" E, 04.04.2024, with flowers, coll. R. Vassilev and K. Bakardzhiev (BG-NMNHS-BOT-5165).

This is a new species for the floristic region of Tundzha Hilly Country. According to Valev (1970) it occurs in numerous floristic regions in Central and Southern Bulgaria, mostly in mountainous areas.

***Aristolochia pallida* Willd.** (Aristolochiaceae)

Specimen examined: Sakar Mt., in a mixed deciduous forest near Vladimirovo village, Topolovgrad Municipality, 170 m a.s.l., 42°07'15.4" N and 26°07'46.6" E, 16.04.2025, with flowers, coll. R. Vassilev and K. Bakardzhiev (BG-NMNHS-BOT-5534).

New species for the Tundzha Hilly Country floristic region. It has been registered already in Forebalkan, Stara planina, Sofia region, Rila, Struma valley, Eastern Rhodopes (Asenov 1970), Southern Black Sea coast, Southern Pirin, Slavyanka, Mesta valley, Central Rhodopes, Thracian Lowland, Vitosha and West Frontier mountains (Assyov et al. 2012, Stoyanov et al. 2022).

***Geranium macrorrhizum* L.** (Geraniaceae)

Specimen examined: Sakar Mt., Golemiya Zdravets locality, Topolovgrad Municipality, 700 m a.s.l., 41°59'59.6" N and 26°19'33.9" E, 22.04.2023, without generative organs, coll. A. Petrova and R. Vassilev (BG-NMNHS-BOT-5497); Sakar Mt., Golemiya Zdravets locality, Topolovgrad

Municipality, 700 m a.s.l., 41°59'58.9" and 26°19'33.2", 07.05.2025, with flowers, obs. R. Vassilev (PI@ntNet: <https://identify.plantnet.org/k-southeastern-europe/observations/1026945383>).

This species is quite common in the mountainous regions in Bulgaria but has not been reported until now from the floristic region of Tundzha Hilly Country, nor from the neighbouring floristic regions in South-eastern Bulgaria (Petrova and Kozuharov 1979, Assyov et al. 2012, Stoyanov et al. 2022).

***Hippocrepis emerus* (L.) Lassen** (syn. *Coronilla emerus* L.) (Fabaceae)

Specimen examined: Sakar Mt., on the outskirts of an artificial pine forest SE from Topolovgrad town, 380 m a.s.l., 42°04'39.0" N and 26°21'03.9" E, 28.04.2025, with flowers, coll. R. Vassilev (BG-NMNHS-BOT-5906).

This thermophilic shrub is known from numerous floristic regions mainly in Southern Bulgaria (Kozuharov 1976, Assyov et al. 2012) but Tundzha Hilly Country has been omitted from the distribution range of the species.

***Mercurialis perennis* L.** (Euphorbiaceae)

Specimen examined: Sakar Mt., near Mramor village, Topolovgrad Municipality, in a deciduous forest, 230 m a.s.l., 42°03'23.6" N and 26°23'27.6" E, 11.04.2024, with fruits, obs. R. Vassilev (PI@ntNet: <https://identify.plantnet.org/k-southeastern-europe/observations/1025468804>).

A widespread species in other floristic regions of Bulgaria but not recorded so far from Tundzha Hilly Country (Kuzmanov 1979, Assyov et al. 2012).

***Osyris alba* L.** (Santalaceae)

Specimen examined: Sakar Mt., near Mramor village quarry, Topolovgrad Municipality, on steep calcareous rocky

slope, 25.05.2024, with flowers, obs. M. Nenchev; same location, 170 m a.s.l., 42°01'50.4" N and 26°25'00.4" E, 17.07.2024, without generative organs, coll. R. Vassilev (BG-NMNHS-BOT-5904); same location, 25.05.2025, with flowers, coll. R. Vassilev and B. Filipova (BG-NMNHS-BOT-6066).

So far, this Mediterranean floristic element has been known only from Black Sea coast (Southern), Struma valley (Southern), Rhodopes (Eastern) and Strandzha floristic regions (Kuzmanov and Kozhuharov 1966, Assyov et al. 2012). This is the first report of the taxon for Tundzha Hilly County. The plant was first found by M. Nenchev in May 2024. Later in the same year, more than 100 vegetative stems were counted by R. Vassilev in an area of 80 m². In the spring of 2025 the authors found out that all the stems of the population produce male flowers. It can be assumed that they all belong to one male individual.

Pulsatilla montana (Hoppe) Rchb. (Ranunculaceae)

Specimen examined: Sakar Mt., along the tarmac road from Topolovgrad to Vishegrad peak, Polyanata locality, Topolovgrad Municipality, 750 m a.s.l., 42°00'04.5" N and 26°19'13.4" E, 14.04.2018, with fruits, coll. R. Vassilev and K. Bakardzhiev (BG-NMNHS-BOT-5163); Sakar Mt., near Haydushkata Dupka natural monument, Harmanli Municipality, 700 m a.s.l., 41°59'53.5" N and 26°14'52.1" E, 20.03.2020, with flowers, obs. V. Trifonov (pers. comm.); Sakar Mt., Golemiya Zdravets locality, Topolovgrad Municipality, 750 m a.s.l., 41°59'56.9" N and 26°19'30.0" E, 22.04.2023, with fruits, coll. A. Petrova and R. Vassilev (BG-NMNHS-BOT-5498); Svetiilyski Heights: in grassy places in young forests in their eastern half, Yambol region, 02.05.1943,

with flowers, coll. D. Jordanov (SO 23350, sub *Anemone pulsatilla* L.).

The floristic region of Tundzha Hilly Country has not been included in the distribution range of *Pulsatilla montana* so far (Jordanov and Kozhuharov 1970, Assyov et al. 2012, Stoyanov et al. 2022). Our observations as well as an older specimen from the herbarium of Sofia University demonstrate that the species is distributed in that floristic region.

Species confirmed for the Tundzha Hilly Country floristic region

Acer monspessulanum L. (Sapindaceae)

Specimen examined: Sakar Mt., near Ustrem monastery, Topolovgrad Municipality, on calcareous slope, 150 m a.s.l., 42°02'00.3" N and 26°25'37.1" E, 15.04.2018, with fruits, coll. R. Vassilev (BG-NMNHS-BOT-5510).

Cheschmedjiev (1967) reported *Acer monspessulanum* var. *rumelicum* Grsb. as new record in Sakar Mt. Curiously, Palamarev (1979) mentioned the region of Topolovgrad in the distribution range of *Acer monspessulanum* var. *commutatum* (C. Presi) Palam., but erroneously placed this record in Thracian Lowland. The species distribution in Tundzha Hilly Country was further omitted in later sources (Delipavlov et al. 2003, Kozhuharov 1992, Stoyanov et al. 2022). Thus, we confirm that the species indeed occurs in this floristic region.

Digitalis grandiflora Mill. (Plantaginaceae)

Specimen examined: Sakar Mt., along the asphalt road to Vishegrad peak, in deciduous forest, with flowers, 05.07.2017, obs. K. Bakardzhiev (PI@ntNet: <https://identify.plantnet.org/k-southeastern-europe/observations/1029615440>); Sa-

kar Mt., by a dirt road in deciduous forest, with flowers, 550 m a.s.l., 42°01'24.8" N and 26°19'01.9" E, 24.06.2025, obs. G. Gerdzhirov (pers. comm.).

This mostly mountainous species has been considered widespread in Bulgaria (Asenov 1995, Delipavlov et al. 2003) but in the Conspectus of Bulgarian vascular flora (Assyov et al. 2012) the region of Tundzha Hilly Country has been omitted from its distribution range.

Tephroseris papposa (Rchb.) Schur subsp. ***papposa*** var. ***papposa*** (syn. *Senecio procerus* var. *montanus* Stoj.) (Asteraceae)

Specimen examined: Sakar Mt., clearing and mixed forest edge on Golemiya Zdravets locality, Topolovgrad Municipality, 750 m a.s.l., 41°59'55.9" N and 26°19'29.2" E, 24.05.2025, with flowers, coll. R. Vassilev (BG-NMNHS-BOT-6236).

The species has been indicated for Sakar Mt. by Podpéra (1902), sub *Senecio procerus* Griseb. Jordanov (1944) is also listing it (sub *Senecio procerus* (Griseb.) Cuph. var. *montanus* Stoj.) among the herbaceous plant species forming the undergrowth on the high slopes of Sakar Mt. In more recent Bulgarian plant identification keys (Andreev et al. 1992, Delipavlov et al. 2003, Stoyanov et al. 2022) as well as in the Conspectus of Bulgarian vascular flora (Assyov et al. 2012) the Tundzha Hilly Country floristic region has been omitted from the distribution range of the species. Our material is identified following Vladimirov (2012).

Vitis vinifera L. (syn. *Vitis sylvestris* C.C.Gmel.) (Vitaceae)

Specimen examined: Sakar Mt., near Mramor village quarry, Topolovgrad Municipality, along the asphalt road, 140 m a.s.l., 42°01'59.8" N and 26°25'32.7" E, 22.04.2023, without generative organs,

coll. R. Vassilev and K. Bakardzhiev (BG-NMNHS-BOT-5499).

This liana species is considered widespread throughout the country by Delipavlov et al. (2003), especially in floodplain (longoz-type) forests along swamps and rivers (Gramatikov 1992). However, Jordanov and Peev (1979) and Assyov et al. (2012) are limiting its distribution to the floristic regions of Black Sea coast, North-Eastern Bulgaria, Stara planina (Central, Eastern), Rhodopes (Western, Eastern), Struma valley (Southern), Mesta valley, Thracian Lowland and Strandzha. We confirm the distribution of the species in Tundzha Hilly Country floristic region.

Species new for the Thracian Lowland floristic region

Asplenium adiantum-nigrum subsp. ***onopteris*** (L.) Heubl. (syn. *Asplenium onopteris* L.) (Aspleniaceae)

Specimen examined: Sakar Mt., near Golyamata Zvezda road junction, Harmanli Municipality, in cedar plantation, 240 m a.s.l., 41°58'12.9" N and 26°00'0.9" E, 08.02.2024, without generative organs, coll. R. Vassilev and B. Assyov (BG-NMNHS-BOT-5162).

The taxon is new for the Thracian Lowland floristic region. According to Ahtarov and Jordanov (1963), it has been registered already in Stara planina (Central, Eastern), Sredna gora, Pirin (Northern), Rhodopes (Central, Eastern) and Strandzha.

Legousia pentagonia (L.) Druce (Campanulaceae)

Specimen examined: Sakar Mt., near Studena village, Svilengrad Municipality, in fields, 370 m a.s.l., 41°55'37.1" N and 26°23'18.0" E, 30.05.2020, with flowers, coll. A. Petrova and R. Vassilev (BG-

NMNHS-BOT-5501).

New floristic region for the distribution of this species, included in the Red List of Bulgarian vascular plants (Petrova and Vladimirov 2009) as Vulnerable. As East-Mediterranean element it is known so far only from the Eastern Rhodopes floristic sub-region (Assyov et al. 2012).

***Paragymnopteris marantae* (L.) K. H. Shing** (syn. *Cheilanthes marantae* (L.) Domin.) (Pteridaceae)

Specimen examined: Sakar Mt., in rock crevices, on steep slope on the right bank of the Tundzha River, 120 m a.s.l., 41°56'08.4" N and 26°33'18.8" E, 04.05.2025, without generative organs, coll. R. Vassilev (BG-NMNHS-BOT-6047); Sakar Mt., in rock crevices, in the foothills of Mount Paleokastro, 270 m a.s.l., 42°04'22.7" N and 26°17'47.7" E, 03.06.2025, with sporangia, obs. G. Gerdzhikov and B. Assyov.

This is the first report of the species for the Thracian Lowland floristic region. The first locality is situated within the territory of Zhdrelo to na Reka Tundzha protected site (Register of protected areas in Bulgaria 2025). So far the species has been registered in Central Stara planina, Rila, Pirin, Belasitsa, Eastern Rhodopes, Strandzha, Eastern Stara planina, West Frontier Mountains, Southern Struma valley, Western and Central Rhodopes (Ahtarov and Jordanov 1963, Assyov et al. 2012).

Further research needed

***Campanula rotundifolia* gr.** (Campanulaceae)

Specimen examined: Mount Dervishka mogila, Svilengrad Municipality, on metamorphic rocks, 650 m a.s.l., 41°54'53.7" N and 26°21'01.7" E, 30.05.2020, with flowers, coll. R. Vassilev and A. Petrova

(BG-NMNHS-BOT-5500); Sakar Mt. on the rocks of Dervishka mogila, silicate, 07.10.1962, with flowers and fruits, coll. N. Vyhodtsevsky (SOM 105859, sub *Campanula rotundifolia* L.); Sakar Mt. – along rock cracks in the upper parts of Dervishka Mogila, Svilengrad region, 26.05.1963, with flowers, coll. D. Jordanov (SO 91294, sub *Campanula rotundifolia* L.).

During a field visit to Mount Dervishka Mogila we observed an interesting plant belonging to the *Campanula rotundifolia* group. This is a taxonomically difficult group, with seven species in the Bulgarian flora (Anchev 2012). As this species complex is known to have a more or less mountain distribution in Bulgaria, it is our belief that its discovery at relatively low altitude prompts a dedicated study including genetic analysis of DNA sequences.

Conclusion

Floristic data are reported for 16 taxa, new or confirmed for the Tundzha Hilly Country and Thracian Lowland floristic regions. Among them are four species with mainly Mediterranean range: *Acer monspessulanum*, *Aristolochia pallida*, *Legousia pentagonia* and *Osyris alba*. The three mainly mountain species: *Aconogonon alpinum*, *Aethionema saxatile* and *Geranium macrorrhizum* have isolated locations in those low mountain areas.

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Cultural ecosystem services of the protected parks of Ukraine during the war

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Thematic field: Forest policy and key challenges

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Abstract

In this study, we determined the effectiveness and perceived satisfaction with cultural ecosystem services in Ukraine during the war in six protected areas. The aim of the study was to determine the importance and effectiveness of eight cultural ecosystem services during the war for visitors to six protected parks in four regions of Ukraine. The following methods were used: field survey (route), analytical, systematisation, sociological, socio-psychological and statistical; the Likert psychometric scale was used to survey visitors to protected parks. The data were analysed using descriptive statistics. In all parks, female respondents predominated, their participation ranged from 52.8 % to 69.2 %. Park visitors were mainly young people aged 16–30 (from 30.6 % to 77.5 %) and people aged 51–64. During the war, visitors to all studied parks noted the importance and effectiveness of cultural ecosystem services, such as recreation, aesthetics, and natural history. The age groups 16–30 and 51–64 were the most in need of such services. The effectiveness and importance of the service of inspiration were confirmed, while spiritual enrichment scored the lowest among all age groups in the four parks. Almost one third of visitors in four parks offered suggestions for improving environmental quality, with the greatest demand being for enhanced landscaping and an increase in the number of trees. The quality of vegetation and the location of the parks influenced overall visitors' satisfaction. The results of this research can be used to improve environment quality through urban green spaces and to address community needs via cultural ecosystem services provided by the studied parks.

Key words: cultural heritage, park users, protected areas, recreation, visitor surveys.

Introduction

The aesthetic quality of the landscape and the presence of special wildlife habitats create positive impacts on people (Havinga et al. 2024). However, rapid urbanisation has caused conflicts between blue-green spaces and other urban infrastructure, which challenges urban planning efforts aimed at improving the well-being of city residents (Huang et al. 2024). Ukraine has been experiencing war for over three years, which is bringing economic and social changes, reducing the area of green spaces and worsening the ecological condition of existing ones. Therefore, one of the intangible benefits that the environment can currently provide to people is cultural ecosystem services (CES). These are a crucial component of ecosystem services (ES), which represent social and humanitarian values (Yang and Duan 2024). They contribute to human well-being and, simultaneously, CES and Blue carbon ecosystems also play a significant role in mitigating global climate change (Quevedo and Kohsaka 2024). Urban green spaces facilitate the interaction of residents with blue-green infrastructure and play a key role in strengthening the human–nature connections, as they offer diverse opportunities for direct contact with the natural environment.

They are reliable providers of CES, the intangible benefits that arise from interactions between people and nature (Grzyb 2024, Li et al. 2024). The diverse cultural ecosystem services provided by urban parks have a direct impact on human health and well-being (Song et al. 2023).

CES improve mental and physical health by providing access to blue-green spaces through aesthetic and recreational experiences that vary with seasonality (Huang et al. 2024). Recreation

in green and blue spaces contributes to the well-being of urban residents in multiple ways, and the nature of the impact depends both on the characteristics of the space and on those of the visitors (Grzyb 2024). The delivery of CES benefits is significantly influenced by elements of the natural landscape (Li et al. 2024). Assessing the motivations and perceived benefits associated with CES is important, especially in areas with conflicting interests (Pinto et al. 2024).

Protected areas provide invaluable non-market ES, drawing increasing attention to their cultural dimensions (Gan et al. 2024). Parks and protected areas are crucial for delivering both tangible and intangible benefits to society, derived from healthy ecosystems (Ferguson et al. 2023). Understanding the relationship between the CES, provided by parks and the characteristics of different visitor groups, can contribute to the public's willingness to engage with nature, while the effective communication of CES values can support the protection and enhance park ecosystems (Song et al. 2023).

The purpose of the study was to identify the perceived importance and effectiveness of eight cultural ecosystem services during the war, as experienced visitors to six protected parks across four regions of Ukraine.

Materials and Methods

The study was conducted in six protected parks during the first half of the day on weekends in August 2024: the 'Olexandria' State Dendrological Park (DP) of the National Academy of Sciences of Ukraine, the 'Horodnytskyi' Park-Monument of Landscape Art (PMLA), the 'Myklukho-Maklai' PMLA, the 'Shatskyi'

National Nature Park (NNP, arboretum), the 'Bereznivskiy' National Dendrological Park, and the 'Hamarnia' Landscape Preserve (LP, arboretum).

The NNP Shatskyi (arboretum) covers an area of 48,977 ha, of which 22,882.6 ha are owned by the park. It includes different zones: protected zone, zone of regulated recreation, zone of stationary recreation, economic zone. The park's territory mostly includes typical ecosystems of the Western Polissya region: lake, forest, swamp, and meadow ecosystems (Shatsky National Nature Park 2025). Pine stands (*Pinus sylvestris* L.) cover most of the territory. The NNP Shatskyi (arboretum) covers 1.5 ha and contains 147 species of woody plants, 109 of which are rare.

The DP 'Bereznivskiy' occupies an area of 29.5 ha, where 750 species, varieties, and cultivars of woody plants could be found (Oleksiichenko et al. 2019). The following plant collections are represented: 'Forests of the Plains of Ukraine', 'Carpathians', 'Crimea', 'Siberia', 'Far East', 'Central Asia', 'North America', 'China and Japan', 'Rosarium', 'Syringarium', 'Birch Grove', 'Garden of Philadelphus', 'Willows', and other landscape compositions. The park serves as an educational centre. A cascade of fountains was constructed within the territory of the DP 'Bereznivskiy', but currently it is not working.

The PMLA 'Horodnytskyi' was established in the 1890s by Y. K. Czartoryski on the banks of the Sluch River. The park covers an area of 21 ha, on the right-bank part of the park (9.8 ha) deciduous tree species aged 90-130 prevail, while on the left-bank part (11.2 ha), there are mostly coniferous plantations of *Pinus sylvestris*. In total, 52 species of woody plants grow in the park. There is a water spring, a dance floor, a fountain, which is currently in need of restoration.

Three parks – the 'Myklukho-Maklai'

PMLA, the LP 'Hamarnia' (arboretum) in the Zhytomyr region, and the 'Olexandria' State DP in the Kyiv region – have been affected by the RAA.

The 'Myklukho-Maklai' PMLA covers an area of 29.6 ha, has three lakes, children's playgrounds and sports grounds, an open-air theatre, recreational areas, a fountain, cafes, a gazebo with a transparent roof for conducting masterclasses, sculptures, monuments, as well as a natural spring (Malyn City Council 2025). The park's forest ecosystems are dominated by *Quercus robur* L. (31 %) and *Pinus sylvestris* (20 %). There are a total of 66 species and forms of woody plants, with native species prevailing. The group of introduced species is small. The following types of garden and park landscapes are represented: forest (58.8 %), park (28.3 %), meadow (8.9 %), regular park and its elements (1.0 %), and a garden (3.0 %) (Markov and Vyshnevskiy 2013).

The LP 'Hamarnia' comprises three parts: the first (36.0 ha), which includes an arboretum and the Myklukho-Maklai Historical and Local History Museum; the second (300 ha) – a forest with deciduous and coniferous tree species; the third part (795 ha) – the Malynske water reservoir. A significant area is covered by the 100–50-year-old *Pinus sylvestris* and *Quercus robur*, 100–10-year-old *Betula pendula* Roth. and *Alnus glutinosa* (L.) Gaertn. trees. In total, there are 134 species of woody plants, among which deciduous species prevail (82 %).

The 'Olexandria' State Dendrological Park of the National Academy of Sciences of Ukraine was established at the end of the 18th century (1788) by the Branicki family. The arboretum is protected as a National Heritage Site. The current area of the park is 400.7 ha. The historical part is 201.5 ha, where unique natural and

artificial historical landscapes have been preserved. There are three valleys with cascades of ponds, hydraulic structures, and small architectural forms of the late 18th – 19th centuries. The park's centuries-old oak forest (50 ha), in which almost 2000 specimens of *Quercus robur* over 200-year-old grow, has scientific, structural and artistic value. Since the end of the 18th century, the 'Olexandria' park has become one of the first centres of plant introduction in Ukraine. Currently, there are 20 trees of various species at age of over 200. The park includes three collection and demonstration areas: 'Syringirium' (67 species and varieties), 'Cotineretum' (276 species and cultivars) and 'Rosarium' (280 varieties of roses). There is a history museum and a scientific and educational centre (Galkin 2012).

The survey followed the ethical standards while working with people: the research was conducted in accordance with the principles of the Helsinki Declaration of 1975, revised in 2013 (WMA 2013). The eight most common CES (aesthetics, recreation, knowledge of nature, social relations, cultural heritage, inspiration, sense of place, spiritual or religious enrichment) were selected and an anonymous survey was conducted taking into account their perceived importance and effectiveness among casual park visitors. A total of 247 respondents from the six parks across four regions of Ukraine (Volyn, Rivne, Zhytomyr, and Kyiv regions) were surveyed. Respondents from various age categories participated in the survey: 16–30, 31–40, 41–50, 51–64, and 65 years and over.

The questionnaire comprised four sections. In the first section, respondents provided basic socio-demographic information. The second and third sections were aimed at assessing the subjective importance and the perceived level of satisfac-

tion (effectiveness) of cultural ecosystem services (CES), which were measured on a five-point Likert scale: from 1 – extremely unimportant to 5 – very important, and from 1 – extremely dissatisfied to 5 – extremely satisfied, respectively. The assessment of importance and effectiveness covered eight CES categories: aesthetic value, recreational function, knowledge of nature, social connections, cultural heritage, inspiration, sense of place and spiritual or religious enrichment. The last section contained an open-ended question, in which participants could suggest measures to improve the park environment (Gai et al. 2022b). The method of analysis of importance was used to interpret the results. Data processing was carried out using IBM SPSS Statistics version 29.0. Likert scale scores were converted into numerical values to quantify importance and satisfaction across all CES categories. The reliability of the questionnaire was tested using Cronbach's alpha coefficient.

Study area

The study was conducted in six protected areas of Ukraine, including two dendrological parks (DP), two park-monuments of landscape art (PMLA), the arboretum within a national nature park (NNP) and a landscape preserve (LP) (figs 1 and 2).

Results and Discussion

Since February 2022, as a result of the Russian armed aggression (RAA), more than 21.9 % of protected areas in Ukraine (812 protected areas with a total area of 0.9 million hectares) have been affected, and 19.5 % (514 protected areas, 0.8 million hectares) remain occupied (WWF

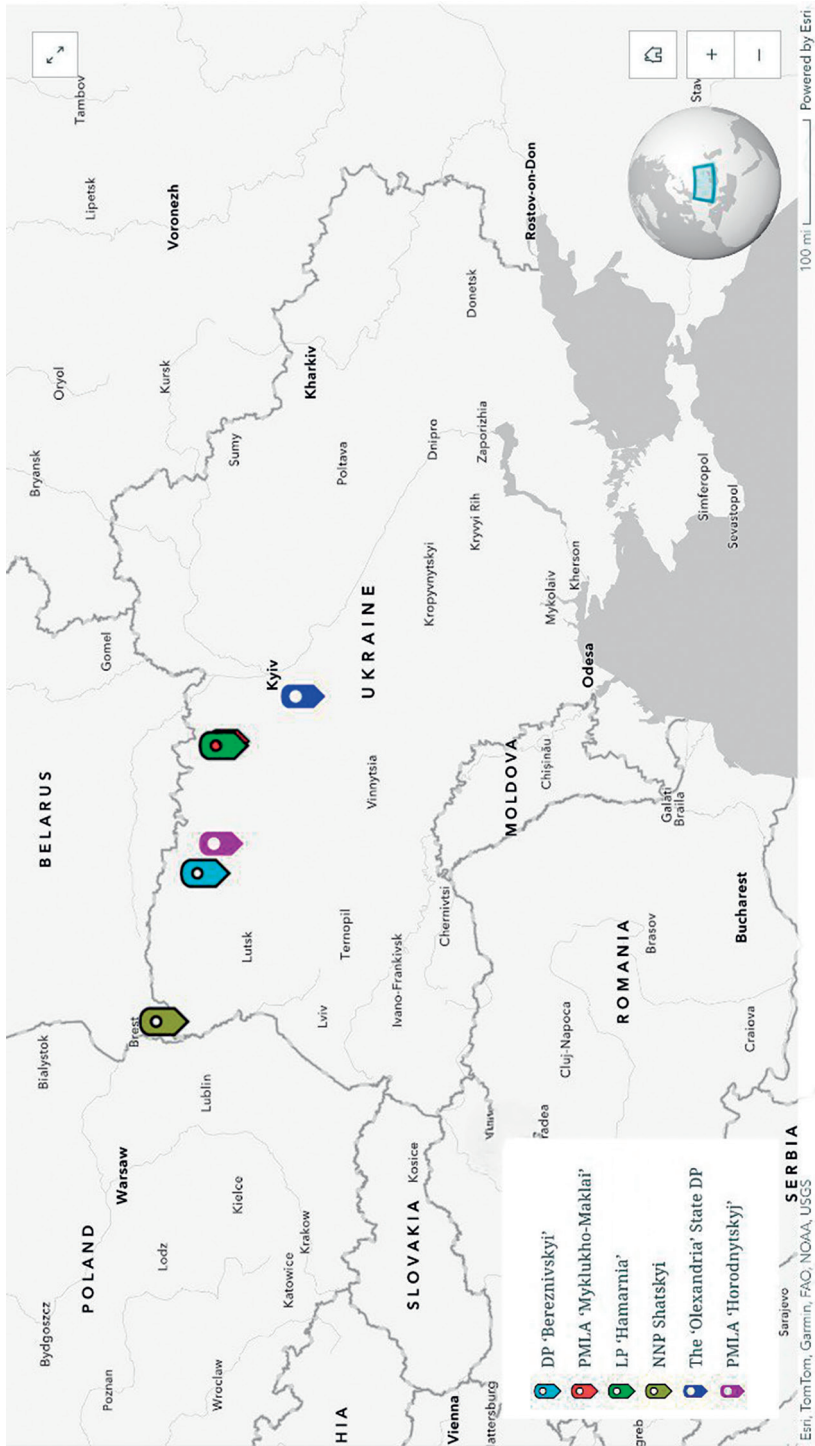


Fig. 1. Protected parks of Ukraine. Source: internet service ArcGIS Online, 2025.

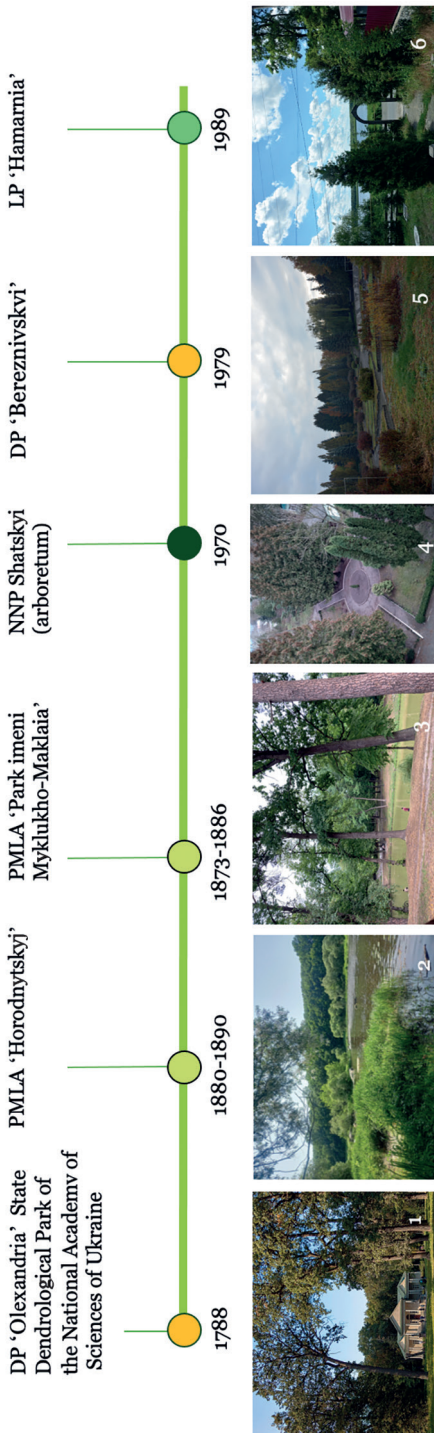


Fig. 2. Representative views of cultural ecosystem services in protected parks in Ukraine.

Ukraine 2023). Biodiversity is lost through warfare (Hanson 2018). The loss of wildlife habitat in war zones depends on the scale, intensity and technology of the conflict. Munitions and chemicals also have direct and indirect, as well as immediate and residual impacts on wildlife habitat (Dudley et al. 2002). This leads to significant changes in population and land use and stimulates an increase in plantings and preservation of existing protected areas, including parks. To provide the variety of user groups and relatively even distribution of parks throughout the research area, and to identify the importance and effectiveness of CES of protected parks, six protected areas in four different regions of Ukraine were selected. Various categories of protected parks were chosen (dendrological parks, park-monuments of landscape art, arboretum within the national natural parks and landscape preserve), which are located in relatively safe territories (north-western regions of Ukraine – Volyn, Rivne regions and the western part of Zhytomyr region of Central Ukraine), and those that have been by subjected to the RAA (Northern part of Central Ukraine – Zhytomyr, Kyiv regions). The NNP Shatskyi in Volyn region, DP 'Bereznivskyi' in Rivne region and the PMLA 'Horodnytskyi' in the Zhytomyr region were located in relatively safe regions.

In all studied parks, the ratio of male and female respondents was not uniform. Female visitors dominated in all parks, with their proportion ranging from over 52.8 % in the DP 'Bereznivskyi' to 69 % in the PMLA 'Horodnytskyi' (Table 1).

In four parks, the proportion of men who visited those parks was less than 40 %. However, as late as in the end of 2022, men were more common in the Sovky Park of the Kyiv region (Dzyba and

Table 1. Demographic and socio-economic characteristics of park visitors, in %.

Categories	Region and parks					
	Volyn		Zhytomyr		Rivne	Kyiv
	NNP Shatskyi	PMLA 'Horodnytskyj'	PMLA 'Park Myklukho-Maklaia'	LP 'Hama-rnia'	DP 'Bereznivskyi'	'Olexandria' State DP
Gender						
Male	41.2	31.0	35.7	31.6	47.2	40.0
Female	58.8	69.0	64.3	68.4	52.8	60.0
Age, years						
16–30	14.3	77.5	52.4	36.8	30.6	23.6
31–40	20.0	17.5	16.7	15.8	13.9	20.0
41–50	22.9	5.0	21.4	15.8	8.3	14.6
51–64	31.4	-	7.1	15.8	30.6	23.6
64+	11.4	-	2.4	15.8	16.6	18.2
Education						
School	2.9	9.5	19.0	2.6	30.6	9.1
College	8.6	11.9	40.5	47.4	22.2	25.4
University	88.5	78.6	40.5	50.0	47.2	65.5
Monthly income (UAH)						
<10,000	62.8	78.6	71.4	71.1	80.6	58.2
10,000–20,000	34.3	19.0	21.4	23.7	19.4	18.2
20,000–30,000	2.9	2.4	2.4	2.6	-	12.7
>30,000	-	-	4.8	2.6	-	10.9
Absent income	-	-	-	-	16.7	-
Occupation						
Service-/factor workers	-	16.7	21.4	21.1	2.8	20.0
Government/corporate and technical staff	74.2	45.2	42.9	44.7	41.6	45.4
Pensioners	20.0	-	2.4	10.5	27.8	18.2
Pupils/students	2.9	28.6	30.9	23.7	27.8	10.9
Unemployed	2.9	9.5	2.4	-	-	5.5
Place of residence						
Local residents	85.7	92.8	81.0	65.8	72.2	74.5
Displaced persons	2.9	4.8	-	-	5.6	9.1
Passers-by	11.4	2.4	19.0	34.2	22.2	16.4

Saveliev 2023). In five parks, the majority of respondents were young people, whose share ranged from 23.6 % (the 'Olexandria' State DP) to 77.5 % (PMLA 'Horodnytskyj'), except for the NNP Shatskyi, where visitors were mainly people aged 51–64 (31.4 %), which is explained by the specifics of the park.

Most respondents had higher education and were employed in governmental, corporate, or technical professions. The share of this group in the NNP Shatskyi was the highest – 74.2 %. In the other five parks their share was almost uniform from 41.6 % in the DP 'Bereznivskyi' to 45.4 % in the 'Olexandria' State DP and

also was independent of the geographic region. There was also a certain share of service-oriented workers, pupils and students.

In all parks, the monthly income of the majority of visitors (from 58.2% in the 'Olexandria' State DP to 80.6 % in DP 'Bereznivskiy') was not high, which shows the accessibility of cultural ES regardless of the region. Small number of visitors were unemployed, which occurred in three regions.

In six parks, visitors were mostly local residents, while in four parks a small proportion of visitors were displaced persons, ranging from 2.9 % in NNP Shatskyi to 9.1 % in the 'Olexandria' State DP. These parks are located in relatively dangerous regions of Ukraine, where people moved at the beginning of the war in 2022. Also, the respondents of the parks included passers-by, a significant number of whom, 34.2 and 22.2 %, were noted in two parks of two regions, respectively, the LP 'Hamarnia' (Zhytomyr Region) and the DP 'Bereznivskiy' (Rivne Region), which is explained by the location of the parks

in relation to residential areas. In general, everything to some extent indicates the diversity of people visiting parks.

Tables 2–7 present both the Cronbach's alpha coefficients of each CES category and the respondents' ratings of importance and effectiveness across the eight CES categories. The Cronbach's alpha coefficient for most categories in six parks was higher than 0.6, except for two elements in the PMLA 'Myklukho-Maklai' and three elements in the LP 'Hamarnia', which may indicate a lack of consistency in the responses. An alpha of 0.65–0.80 is considered 'adequate' for a scale used in human dimension research (Vaske et al. 2017).

The results of the analysis of variance confirmed significant gaps between the perceived importance and effectiveness of CES. Zoderer et al. (2016) note that people's cultural background is an important factor in determining their perception of the importance of CES. However, the main landscape types have a greater impact on the sociocultural assessment of respondents in the ES categories.

Table 2. Cultural ecosystem services of NNP Shatskyi.

Cultural ecosystem services		Importance			Satisfaction		
		Average	Mean square	Cronbach's alpha	Average	Mean square	Cronbach's alpha
A	Aesthetics	4.77	0.490	0.831	4.51	0.781	0.824
B	Recreation	4.49	0.781	0.832	4.57	0.917	0.809
C	Knowledge of nature	4.29	1.073	0.826	4.40	0.775	0.840
D	Social relations	3.46	1.094	0.820	3.71	0.860	0.808
E	Cultural legacy	3.60	1.241	0.792	3.83	0.954	0.810
F	Inspiration	4.14	1.033	0.806	4.34	0.804	0.820
G	Sense of place	3.66	1.282	0.794	4.09	0.951	0.832
H	Spiritual or religious enrichment	3.11	1.231	0.803	3.40	0.976	0.817
Total (<i>n</i> = 35)		3.94	1.028	0.813	4.11	0.877	0.820

Table 3. Cultural ecosystem services of DP 'Bereznivskiy'.

Cultural ecosystem services		Importance			Satisfaction		
		Average	Mean square	Cronbach's alpha	Average	Mean square	Cronbach's alpha
A	Aesthetics	4.56	0.695	0.742	4.11	0.820	0.549
B	Recreation	4.44	0.773	0.706	4.31	0.889	0.490
C	Knowledge of nature	4.47	0.774	0.730	4.31	0.749	0.612
D	Social relations	4.19	1.009	0.673	4.17	0.811	0.577
E	Cultural legacy	3.81	1.117	0.731	4.28	0.815	0.658
F	Inspiration	4.39	0.766	0.756	4.11	0.887	0.573
G	Sense of place	4.03	1.055	0.750	4.06	0.893	0.570
H	Spiritual or religious enrichment	3.44	1.120	0.769	3.75	0.967	0.626
Total (<i>n</i> = 36)		4.17	0.914	0.732	4.14	0.854	0.582

Table 4. Cultural ecosystem services of the PMLA 'Horodnytskyj'.

Cultural ecosystem services		Importance			Satisfaction		
		Average	Mean square	Cronbach's alpha	Average	Mean square	Cronbach's alpha
A	Aesthetics	4.40	0.672	0.640	3.73	1.012	0.690
B	Recreation	4.58	0.675	0.620	4.10	0.928	0.708
C	Knowledge of nature	4.30	0.648	0.661	4.13	0.939	0.730
D	Social relations	3.95	0.876	0.615	3.78	1.074	0.697
E	Cultural legacy	4.20	0.823	0.668	3.65	1.122	0.724
F	Inspiration	4.25	0.776	0.606	3.90	0.928	0.744
G	Sense of place	4.23	0.768	0.619	3.95	0.959	0.749
H	Spiritual or religious enrichment	3.78	1.025	0.751	3.83	0.903	0.746
Total (<i>n</i> = 40)		4.21	0.783	0.648	3.88	0.983	0.724

Table 5. Cultural ecosystem services of the PMLA 'Myklukho-Maklai'.

Cultural ecosystem services		Importance			Satisfaction		
		Average	Mean square	Cronbach's alpha	Average	Mean square	Cronbach's alpha
A	Aesthetics	4.29	0.708	0.629	4.29	0.708	0.872
B	Recreation	4.55	0.670	0.687	4.36	0.906	0.865
C	Knowledge of nature	4.19	0.634	0.650	4.21	0.871	0.862
D	Social relations	4.14	0.952	0.652	4.02	1.047	0.872
E	Cultural legacy	4.14	0.926	0.593	4.29	0.835	0.871
F	Inspiration	4.31	0.924	0.565	4.26	0.798	0.853

Cultural ecosystem services		Importance			Satisfaction		
		Average	Mean square	Cronbach's alpha	Average	Mean square	Cronbach's alpha
G	Sense of place	4.12	1.017	0.647	4.12	1.017	0.862
H	Spiritual or religious enrichment	3.21	1.353	0.682	3.64	1.144	0.860
Total ($n = 42$)		4.12	0.898	0.638	4.15	0.916	0.865

Table 6. Cultural ecosystem services of LP 'Hamarnia'.

Cultural ecosystem services		Importance			Satisfaction		
		Average	Mean square	Cronbach's alpha	Average	Mean square	Cronbach's alpha
A	Aesthetics	4.31	0.655	0.686	4.33	0.772	0.832
B	Recreation	4.23	0.777	0.660	4.41	0.751	0.811
C	Knowledge of nature	4.18	0.756	0.583	4.44	0.821	0.768
D	Social relations	3.85	0.961	0.631	4.03	0.986	0.791
E	Cultural legacy	4.36	0.707	0.677	4.36	0.707	0.796
F	Inspiration	4.46	0.942	0.589	4.38	0.907	0.774
G	Sense of place	4.33	0.955	0.564	4.28	0.857	0.770
H	Spiritual or religious enrichment	3.33	1.457	0.687	3.77	1.135	0.789
Total ($n = 39$)		4.13	0.901	0.635	4.25	0.867	0.791

Table 7. Cultural ecosystem services of the 'Olexandria' State DP.

Cultural ecosystem services		Importance			Satisfaction		
		Average	Mean square	Cronbach's alpha	Average	Mean square	Cronbach's alpha
A	Aesthetics	4.49	0.573	0.708	4.69	0.466	0.774
B	Recreation	4.67	0.579	0.727	4.80	0.524	0.787
C	Knowledge of nature	4.15	0.870	0.730	4.62	0.757	0.769
D	Social relations	3.24	1.232	0.732	3.84	0.918	0.756
E	Cultural legacy	4.22	0.832	0.710	4.53	0.604	0.765
F	Inspiration	4.24	0.922	0.682	4.51	0.717	0.758
G	Sense of place	3.65	1.142	0.697	3.96	1.036	0.757
H	Spiritual or religious enrichment	3.16	1.259	0.687	3.76	1.018	0.735
Total ($n = 55$)		3.98	0.921	0.737	4.34	0.612	0.787

The overall effectiveness of CES in four parks (NNP Shatskyi, PMLA 'Myklukho-Maklai', LP 'Hamarnia', the 'Olexandria' State DP) was higher than perceived importance (respectively 4.11, 4.15, 4.25, and 4.34 vs.) (3.94, 4.12, 4.13, and 3.98). Three of these parks are located in areas affected by the RAA. Dzyba and Saveliev (2023) obtained similar results in Kyiv, where overall satisfaction of with CES was higher (4.08 and 4.38) than perceived importance (3.69 and 3.92) of CES, highlighting the impact of the RAA.

In two parks located in relatively dangerous areas – DP 'Bereznivskyi' and PMLA 'Horodnytskyi' – respondents perceived the overall importance of CES (4.17 and 4.21) as higher than its effectiveness (4.14 and 3.88). Similar results were obtained by Gai et al. (2022b), who conducted a study during peacetime in a park in China, where the overall importance (4.299) of the CES perceived by all respondents was higher than satisfaction (3.613). Gai et al. (2022a) noted that the satisfaction by users of the CES of an urban park was generally lower than its importance regardless of style and design, because users had higher expectations of parks.

Elbakidze et al. (2025) examined the impact of the Russian invasion on the relationship between people and nature in three settlements in Kyiv region that were occupied by Russian troops and later liberated by the Ukrainian army, and found that ecosystem services play an important role and influence the emotional and spiritual connection of respondents with their natural environment. Therefore, private gardens and urban green spaces have become crucial elements for post-war recovery, promoting reconnection and psychological healing.

Among the six parks, the main CES

categories that influenced user satisfaction differ. In the 'Olexandria' State DP, PMLA 'Myklukho-Maklai', five categories of the 'supply and demand correspondence zone' were identified: Aesthetics (A), Recreation (B), Knowledge of nature (C), Cultural legacy (E), Inspiration (F). There are three categories in both DP 'Bereznivskyi' and PMLA 'Horodnytskyi', among which two categories are common (average value is greater 4.0): Recreation (B) and Knowledge of nature (C), and while the third category is different: Social relations (D) in DP 'Bereznivskyi' and Sense of place (G) in PMLA 'Horodnytskyi'. LP 'Hamarnia' had six categories: Aesthetics (A), Recreation (B), Knowledge of nature (C), Sense of place (G), Cultural legacy (E), Inspiration (F). NNP Shatskyi included four categories: Aesthetics (A), Recreation (B), Knowledge of nature (C), Inspiration (F). The importance and effectiveness of all these categories were relatively high (Table 2–7).

In each park, different CES categories influenced overall user satisfaction. Recreation (B) had the highest score in four parks, while Aesthetics (A) dominated in two parks, located in relatively safe areas. Similar results were reported by Peng et al. (2024), who found that aesthetic value is one of the CES most closely related to humans and important for national park management. In Kyiv, respondents also highly appreciated Aesthetics (A), Recreation (B), Knowledge of nature (C) (Dzyba and Saveliev 2023). Relaxation in parks during the war allows people to restore their mental state in natural silence.

The psychological well-being of urban and rural residents is partly linked to the proximity of green and blue spaces, street trees, and private gardens (Bratman et al. 2019). Van den Berg et al. (2019) note that green spaces within a 3 km radius provide

more effective recovery than those within 1 km, given sufficiently large green areas. Green spaces can provide a buffer against the negative impact of stressful life events on people's health. Short-term contact with nature, including physical activity and social interaction positively affects mental health (Hartig et al. 2014). Therefore, the mental health benefits can be considered 'co-benefits' alongside other ecosystem services (Bratman et al. 2019).

Peng et al. (2024) noted that visitors to national parks tend to prioritise natural, cultural, and social aesthetic CES. The perceived value of physical and mental health services was the highest (2.44), whereas spiritual services were the lowest (1.86). In Ukraine, visitors of the six parks also rated the category Spiritual lowest in terms of Satisfaction (3.40–3.83). Peng et al. (2024) further observed that different demographic characteristics significantly affect the perception of aesthetic values. However, in contrast to respondents from Chinese parks, visitors to Ukrainian parks evaluated Spiritual (H) as the least important among CES (3.11–3.78, tables 2–7). It can be assumed that the war has influenced spirituality – understood as the search for meaning and purpose in life – by prompting people to reassess their values, beliefs, and place in the world. During the RAA, Inspiration (F) became important for personal development, work, and study. Visitors in all six parks also emphasised the relative importance of this category (4.14–4.46, tables 2–7), indicating its strong contribution to overall satisfaction. In historical parks in China, among 14 CES, Sense of place and Relaxation were reported as most important (Dai et al. 2024). Similarly, in Ukrainian historical parks – the 'Olexandria' State DP, PMLA 'Horodnytskyi', PMLA 'Myklukho-Maklai', and LP 'Hamarnia' – Sense of place (G)

had moderate importance and impact on visitors. The highest score was recorded in LP 'Hamarnia' (4.33), likely because most respondents had lived in the area since childhood, which strengthens their emotional attachment to the landscape.

In urbanised areas, visitors rated cultural heritage and education as most important CES, while aesthetic and recreational services were in highest demand. In moderately urbanised small cities, the demand for Recreation (B) and Social relations (D) was lower (Xia et al. 2024). The studied parks are located in small cities with moderate urbanisation. Social relations (D) were generally rated low (3.24–3.95) except in PMLA 'Myklukho-Maklai' and DP 'Bereznivskyi' (4.14–4.19), indicating that the war did not affect social interactions. CES preferences also depend on stand composition: *Pinus sylvestris* stands emphasise aesthetic and cultural legacy, while in broad-leaved stands favour recreation and intellectual services (Bhatt et al. 2024). Broadleaved mixed-age stands with centuries-old *Quercus robur* provide CES and generate the largest current ES increment (Feshchenko et al. 2023).

In studied parks, respondents were divided into five age groups to compare differences in the perception of the effectiveness (satisfaction) of the eight CES categories among these age groups. Among the respondents, clear differences were observed between younger and older visitors in each park (Fig. 3).

The highest levels of satisfaction were reported by visitors aged 31–40 in LP 'Hamarnia' and PMLA 'Myklukho-Maklai', and by those aged 41–50 in DP 'Bereznivskyi' and PMLA 'Horodnytskyi'. In the NNP Shatskyi, the 51–64 age group recorded the highest scores, whereas in the 'Olexandria' State DP, the most satisfied visitors were aged 64 and older.

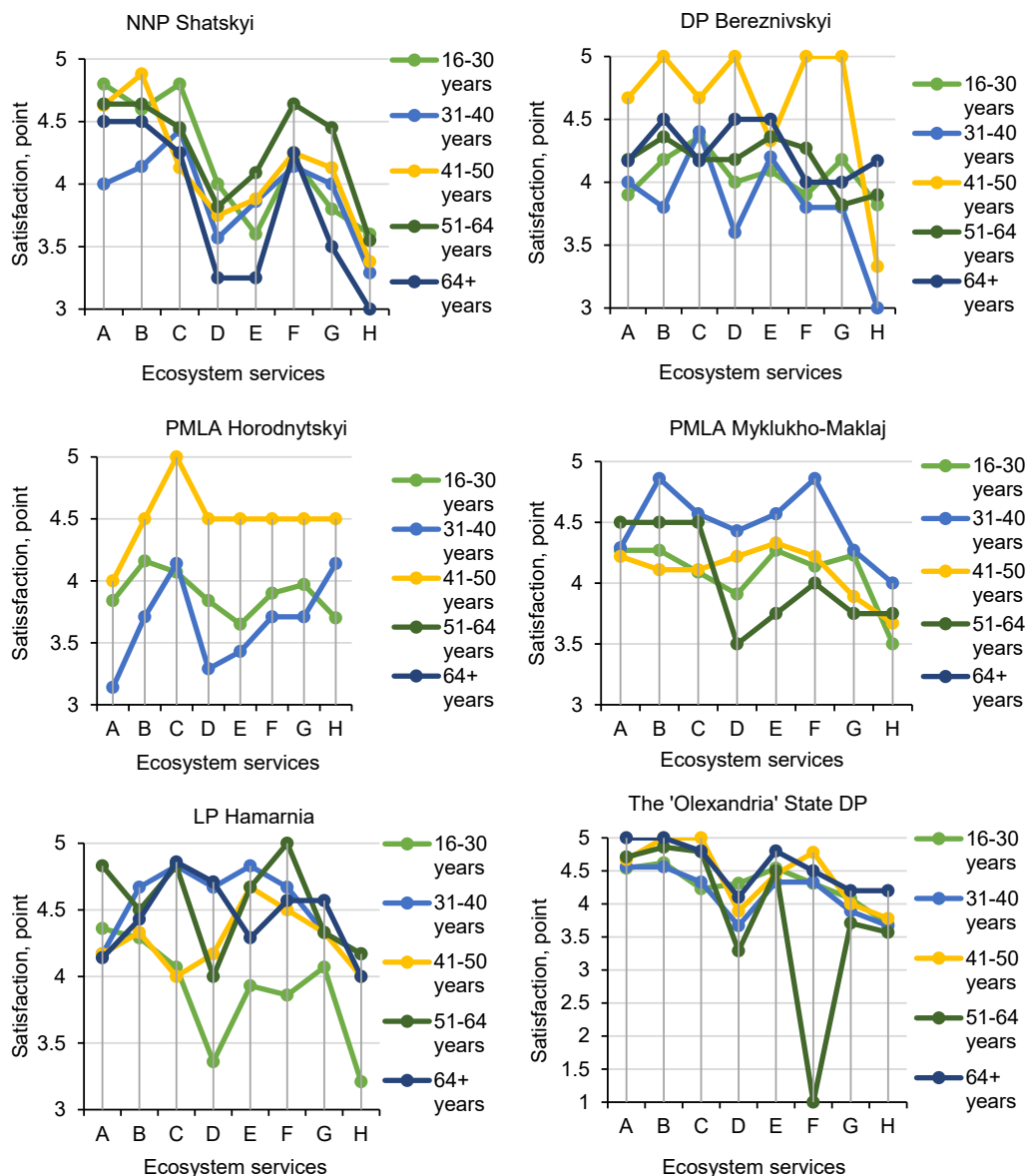


Fig. 3. Satisfaction with cultural ecosystem services by age group in the PMLAs, NNP, DPs, and LP.

Note: A – aesthetics, B – recreation, C – knowledge of nature, D – social relations, E – cultural legacy, F – inspiration, G – sense of place, H – spiritual or religious enrichment.

These results suggest that middle-aged and older people tend to have a more positive perception of CES in parks.

In contrast, the youngest age group (16–30) in all parks reported only moderate satisfaction with CES. They pointed out

the need to improve the quality and diversity of CES. Suggested improvements included: infrastructure improved in several parks and lighting (DP 'Bereznivskiy'), the provision of art and educational events, ecological competitions and quests, art readings (DP 'Bereznivskiy'), creation of information boards with park maps, development of ecological trail (NNP Shatskyi, DP 'Bereznivskiy', LP 'Hamarnia', as well as increased species diversity (NNP Shatskyi, PMLA 'Myklukho-Maklai').

Least satisfied with CES in NNP Shatskyi were visitors aged 64 and over. In PMLA 'Myklukho-Maklai' and the 'Olexandria' State DP, the lowest satisfaction was reported among the 51–64 age group. In DP 'Bereznivskiy' and PMLA 'Horodnytskyi', it was the 31–40 age group, while in LP 'Hamarnia', the youngest respondents aged 16–30 expressed lowest satisfaction (Fig. 3). These results suggest that older visitors (51 and older) may require more accessible infrastructure and a greater number of comfortable resting areas. In contrast, younger respondents (16–30-year-old) tend to expect better-maintained green spaces and more opportunities for active or social leisure within the park.

The type of services offered depends on the physical characteristics of ecosystems (Hegetschweiler et al. 2017). Five parks include reservoirs, except for the DP 'Bereznivskiy', where the fountain cascade is currently non-functional. The presence of water bodies plays a significant role in the effectiveness of cultural ecosystem services. Quevedo and Kohsaka (2024) noted that the provision of CES depends on blue carbon ecosystems, as their degradation leads to the loss or reduction of CES and associated benefits, ultimately affecting community well-being and health.

In the DP 'Bereznivskiy', respondents

indicated a desire to see the restoration of the fountain, while in the NNP Shatskyi they emphasised the need to improve the coastal areas of the Shatsky lakes for recreation, which confirms the opinion of scientists about the importance of reservoirs as integral elements of CES. Coastal ecosystems provide a wide range of ecosystem services and benefits that contribute to local livelihoods, food security, and overall well-being (Himes-Cornell et al. 2018).

Hua and Chen (2019) noted, based on a systematic analysis of community perceptions, that for respondents in South China, the perceived importance of CES of urban rivers was generally higher than their satisfaction levels. We agree with Quevedo and Kohsaka (2024), who emphasise the need to integrate CES into local management and restoration strategies, especially given the growing importance of blue carbon ecosystems for global climate change mitigation. Improving the institutional and legal frameworks for the management, control and regulation of waterscapes and developing ecological restoration options will enhance the long-term values of CES (Barbier et al. 2011). Himes-Cornell et al. (2018) also recommend focusing on expanding valuable ecosystem services and increasing their valuation in underrepresented regions.

From 15.4 % to 94.4 % of respondents in six Ukrainian parks – particularly in areas more significantly affected by the RAA – expressed a desire for improved infrastructure and landscaping. The most frequent requests concerned enhancing aesthetics through the installation of additional benches, waste bins, gazebos, playgrounds, and restrooms (notably in DP 'Bereznivskiy', PMLA 'Myklukho-Maklai', and the 'Olexandria' State DP). Visitors also suggested restoring non-func-

tional fountains (in DP 'Bereznivskiy' and PMLA 'Horodnytskyj'), and creating new flower beds to enhance visual appeal (in PMLA 'Myklukho-Maklai' and 'Olexandria' State DP).

The highest number of proposals was recorded in Shatskyi National Park, where respondents highlighted the need for additional information stands about rare plants and cultural heritage, greater dendrological diversity through the introduction of exotic tree species, creation of photo zones, construction of children's and sports areas, and installation of gazebos and an electronic species indicator. They also expressed a wish for overall park modernisation through contemporary landscape design.

Approximately 5 % of visitors in each park reported being satisfied with the current conditions and did not propose any changes.

Elbakidze et al. (2025) note that post-conflict recovery strategies should prioritise the restoration of ecosystem services and cultural and spiritual connections between people and nature. Analysis of the spatial integration of socio-cultural values will provide an opportunity to predict the consequences of landscape changes and develop recommendations for future landscape planning (Zoderer et al. 2016). In the post-conflict period, which is a critical stage, it is necessary to prioritise biodiversity conservation in recovery and reconstruction policies, natural resource management, protected areas, and the establishment of transboundary peace parks (Hanson 2018).

Conclusions

Protected parks in Ukraine serve as valuable centres for the provision of cultural

ecosystem services. During the RAA, when many people experience stress, these parks provide spaces for restoring well-being and fostering connection with nature. Visitors expressed a preference for secluded natural areas. Overall satisfaction with and perceived importance of CES depend on the influence of RAA. Respondents in parks located in relatively dangerous areas greater importance to CES than their satisfaction with them. Among the eight CES categories, Recreation (B) scored highest in both importance and satisfaction across six parks, while Aesthetics (A) ranked highest in two parks situated in relatively dangerous areas. CES categories, such as Spiritual (H) and Social relations (D), were generally considered less important.

The obtained data can be utilised by communities to enhance park habitats. The CES provided by parks can serve as a foundation for the sustainable management, development, or restoration of blue carbon ecosystems. In the post-war period, taking into account CES, such as recreation and tourism, it is crucial to prioritise the restoration of blue carbon ecosystems within parks. The rehabilitation of damaged or neglected park areas in local communities, with due consideration of cultural values, is highly valued by the public.

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Natural regeneration of post-fire forest areas in Stara Zagora Regional Forest Directorate, Bulgaria

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Thematic field: Forest disturbances and management challenges

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Abstract

The rapid and persistent climate change in recent years has amplified the occurrence and spread of various extreme weather events, causing significant socio-economic, ecological, and cultural damages. Forest fires have become constant threat to human life and major challenge to the resilience of forest ecosystems and their ability to recover after severe disturbances. The focus is on the topic of forest vegetation regeneration as first stage of recovery and adaptation. The study, conducted as part of the FIRE-RES project, focuses on the area affected by one of the most severe wildfires near the village of Dabovo (2007), within the jurisdiction of the Regional Forest Directorate Stara Zagora. Due to its characteristics, the area faces constant threat of extreme fires. The objective of the study is to conduct a scientifically grounded assessment of forest resilience and post-fire recovery capacity. To assess regeneration processes, we established methodology for evaluating regeneration and development of understorey vegetation in forest areas affected by fires. Three areas with different exposures, i.e., different types of habitats, were selected within the burned area, where three transects were laid, covering all altitudes within the fire zone. The inventory in the survey plots was carried out by tree species, origin, coverage, and height. The results demonstrate significant relationships between post-fire regeneration dynamics and the structural and ecological condition of the landscape following the disturbance. The real assessment of regeneration after major disturbances allows precise determination of appropriate forestry measures and the creation of a landscape resilient to various challenges. Fifteen years after the Dabovo fire, natural regeneration remains spatially uneven and incomplete, with pioneer species dominating the sun-exposed slopes, emphasising the need for targeted actions to provide ecosystem recovery.

Key words: adaptation, forest fires, recovery, resilient landscapes.

Introduction

In recent years, there has been a growing frequency of sudden natural disasters that disrupt – and in some cases entirely devastate – the normal course of development in natural ecosystems. Among the primary drivers of these extreme events is the ongoing climate change, specifically rising average temperatures, prolonged droughts, intense precipitation events, and unseasonal cold spells. The significance of such disturbances for forest stands is often substantial and is largely determined by the scale of disruption and the pre-existing condition of forests. Nevertheless, climate change is not the sole factor contributing to the increased vulnerability of natural ecosystems. The intensification of land use – manifested in the expansion of infrastructure and urban areas, the conversion of vegetation for agricultural and pastoral activities, as well as increased human presence in natural sites for tourism, recreation, and sport – has created conditions favourable to the ignition and spread of wildfires, particularly during the warmer and drier months (UNDRR 2017). Anthropogenic climate change is inducing large-scale transformations in forest ecosystems, both directly through changes in temperature and precipitation patterns, and indirectly by altering the natural disturbance regimes (Anderson-Teixeira et al. 2013, McDowell et al. 2020). Frequent and severe wildfires have long-term impacts on forest development patterns and biodiversity (Adámek et al. 2016). Globally, there has been a marked increase in both the number of wildfires and the extent of the damage they cause. According to the European Forest Fire Information System (EFFIS 2025), a total of 8,448 forest fires were recorded across Europe in 2024, affecting an area of 1,684,273 ha.

In Bulgaria alone, 133 fires were registered during the same year, burning a total of 44,745 ha. Increasing attention is being paid by both scientists and practitioners to the issue of limiting the adverse effects of forest fires, particularly in regions, such as those found in South-east European geographic latitudes. Several key focus areas have emerged, including: preventive measures, rapid fire detection, improved strategies for combating ongoing wildfires, and technologies for post-fire recovery and ecosystem adaptation.

Fires exert a destructive impact on forest ecosystems, acting as local ecological catastrophes. In addition to destroying tree stands, wildfires cause degradation across all components of the forest ecosystem – vegetation cover, soil and associated microorganisms, as well as animal communities (Tashev et al. 2003). The ecological consequences of forest fires vary depending on the season, the quantity, condition and distribution of combustible materials, climatic conditions, fire intensity and duration, terrain slope, aspect and elevation, vegetation type, soil properties, and many other factors (Bogdanov 2010). Following such a dynamic disturbance as fire, ecosystem recovery tends to be slow and often incomplete, mainly due to the severity of degradation. Consequently, the affected ecosystems frequently lose their capacity to provide essential ecosystem services – ecological, social, and economic. In particular, disturbances that are both extensive and severe can impair forest recovery processes (i.e., the restoration of forest structure, composition, and function), which are core components of ecosystem resilience (Nikinmaa et al. 2020). Such disruptions may result in successional shifts toward altered forest types or even non-forest ecosystems (Donato et al. 2016, Martínez-Vilalta and

Lloret 2016, Young et al. 2019, Coop et al. 2020). Therefore, the study of post-fire succession, and particularly the regenerative processes of forest vegetation, is of critical importance for ensuring the resilience of natural ecosystems at the landscape level. The main objective of this study is precisely to assess the forest's recovery capacity and the influence of certain determining factors. Although such studies are inherently regional, they often reveal universal ecological patterns (Tashev et al. 2003). Forest regeneration is often hindered by a combination of limited seed availability and the environmental consequences of large-scale, severe disturbances. In particular, drought conditions accompanied by elevated temperatures may significantly obstruct the successful establishment and growth of tree regeneration following fire events (Harvey et al. 2016, Hansen et al. 2018, Davis et al. 2019). The initial reorganisation phase following disturbance is widely recognised as a crucial window for determining future forest dynamics (Seidl and Turner 2022). Forest recovery is strongly constrained by the scale of disturbance. In the case of large burnt areas, it is essential to consider whether viable seed sources remain from surviving trees or adjacent territories, and to what extent the fire has impacted soil properties (Bogdanov 2023).

Wildfires lead to the destruction of vegetation and the degradation of soil, with coniferous forests being most severely affected – especially plantations of Austrian black pine (*Pinus nigra* J.F. Arnold) in the lower altitudinal zones of Bulgaria (Tashev et al. 2003, Bogdanov 2022). Coniferous plantations established outside the natural range of the respective tree species require adapted management and strategies to minimise the negative effects caused by their low resistance to various

biotic and abiotic factors. Their location in the lower vegetation belt is a significant predisposition for the occurrence and spread of forest fires and various calamities (Popov et al. 2016, 2018).

The primary objective of the study is to provide a scientifically substantiated assessment of the capacity of forest ecosystems to regenerate and recover following wildfire disturbances. This involves evaluating the effectiveness and dynamics of natural regeneration processes, identifying key ecological factors influencing recovery, and determining adaptive forest management strategies aimed at enhancing post-fire resilience.

Materials and Methods

The study was conducted in an area affected by a wildfire in 2007 near the village of Dabovo. This area is located in south-central Bulgaria, within the Kazanlak Valley, between the northern slopes of the Sarnena Sredna Gora and the Balkan Mountains (Stara Planina). The area falls within the jurisdiction of the Mazalat State Game Enterprise, managed by the Regional Forest Directorate (RFD) of Stara Zagora (Fig. 1).

The climatic characteristics of the region predispose it to various natural disturbances that can compromise the condition of forest ecosystems and the ecological functions they provide. Average annual temperatures range between 12 and 14 °C, with annual precipitation around 500–600 mm, and prolonged hot and dry periods during summer. The wildfire was recorded on 25 July 2007 and was extinguished five days later. The reported cause of ignition was a short circuit along a railway line, in an area predominantly covered with grasses and shrubs. The

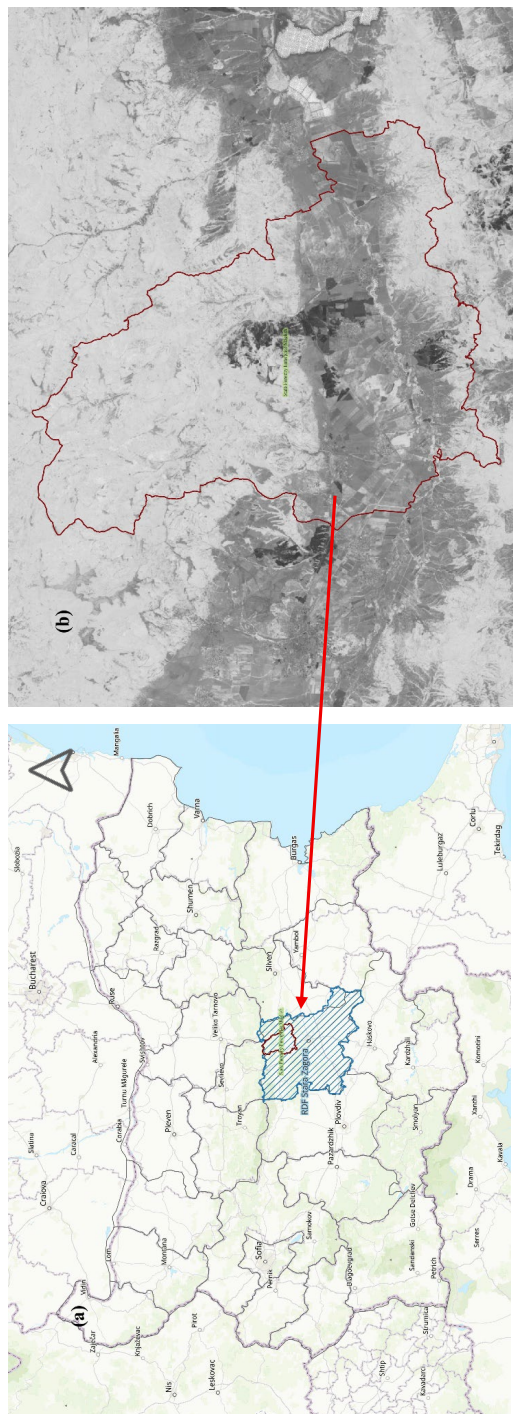


Fig. 1. Location of the studied region: (a) The territorial scope of the Stara Zagora Regional Forestry Directorate is shaded in blue, and the Mazalat State Hunting Enterprise is shown in green. (b) The area affected by the wildfire near the village of Dabovo is outlined in black, illustrating its proportion relative to the total territory of the hunting estate.

fire spread rapidly, resulted in the destruction of 1,370.4 ha of forested land, coniferous Austrian black pine plantations and oak forest stands.

The regeneration assessment was conducted in accordance with the officially adopted Methodology for the Evaluation of Natural Regeneration and Development of Understorey in Forest Areas (EFA 2025). According to this methodology, three linear transects were predetermined, covering three aspects – north-east (NE), south-east (SE), and east (E). Sample plots were established at approximately 20 m intervals, with minor deviations in some locations due to terrain features. The transects measured 307 m, 302 m, and 186 m for the first, second, and third transects, respectively. Along each transect, a total of 40 circular sample plots were established. Each sample plot consisted of two concentric circles with a common centre but different radii: the smaller with a radius of 1.30 m and the larger with a radius of 2.80 m.

The regeneration within the two concentric circles was categorised by tree species, origin, height, and degree of suppression. In the smaller circle, regeneration with a height of up to 1.30 m was recorded, while in the larger circle, individuals exceeding 1.31 m were documented. Only healthy and vigorous regeneration was counted.

The final assessment of regeneration took into account the measured elevation, aspect, and slope – factors which, although indirect, exert a significant influence on the processes involved in the for-

mation of new plant communities.

Regeneration was classified into five categories: very good, good, satisfactory, unsatisfactory, and poor according to Table 1.

Table 1. Regeneration assessment classes concerning the number of seedlings, shoots, and canopy cover.

Level of regeneration	Seedlings, pc·ha ⁻¹			Total shoots, pc·ha ⁻¹	Canopy cover, %
	Total	Shade-tolerant	Heliophilous		
Very good	20,000	23,000	10,500	16,800	81–100
Good	10,000–20,000	15,000–23,000	6,800–10,500	10,900–16,800	61–80
Satisfactory	5,000–10,000	8,000–15,000	3,500–6,800	5,900–10,900	41–60
Unsatisfactory	3,000–5,000	4,500–8,000	2,000–3,500	3,400–5,900	21–40
Poor	3,000	4,500	2,000	3,400	0–20

Note. Column 2 ('Total') is used for assessment in plots where regeneration includes both shade-tolerant and light-demanding tree species. Columns 3 ('Shade-tolerant') and 4 ('Heliophilous') are applied when regeneration consists exclusively of shade-tolerant or exclusively of light-demanding species, respectively.

Results

The assessment of the regeneration processes was carried out 15 years after the disturbance. In the first transect, which is located on a north-eastern aspect, 15 sample plots were established. Regeneration was found to be poor or completely absent in 40 % of the plots. According to the scale provided in the applied regeneration assessment methodology, 53 %

of the plots demonstrated good or very good regeneration. The natural regeneration was dominated primarily by pioneer species such as European hornbeam (*Carpinus betulus* L.), oriental hornbeam (*Carpinus orientalis* Mill.), and European hop-hornbeam (*Ostrya carpinifolia* Scop.). Common beech (*Fagus sylvatica* L.) also played a significant role in the regeneration process. The results are summarised in Table 2 and illustrated in Figure 2.

Table 2. Assessment of natural regeneration in the first transect.

Sample plot	Seedling regeneration			Total shoots, pc·ha ⁻¹	Canopy cover, %	Regeneration estimation	Slope, °	Elevation, m a.s.l.
	Shade-tolerant	Heliophilous	Total, shade-tolerant + heliophilous					
1	24,498	1,884	26,382	9,422	81–100	Very good	27	869
2	3,769	22,613	26,382	13,191	81–100	Very good	31	841
3	1,884	3,769	5,653	16,960	81–100	Very good	32	817
4	0	0	0	0	0–20	Poor	17	793
5	0	5,653	5,653	0	41–60	Satisfactory	30	772
6	22,613	9,422	32,036	13,191	81–100	Very good	22	750
7	0	1,884	1,884	0	0–20	Poor	24	730
8	0	1,884	1,884	0	0–20	Poor	24	715
9	0	1,884	1,884	0	0–20	Poor	24	682

Sample plot	Seedling regeneration			Total shoots, pc·ha ⁻¹	Canopy cover, %	Regeneration estimation	Slope, °	Elevation, m a.s.l.
	Shade-tolerant	Helio-philous	Total, shade-tolerant + heliophilous					
10	1,884	0	1,884	9,422	61–80	Good	16	671
11	3,769	0	3,769	13,191	61–80	Good	38	654
12	39,573	15,076	54,649	0	81–100	Very good	32	628
13	0	0	0	0	0–20	Poor	34	600
14	11,307	0	11,307	0	61–80	Good	26	551
15	0	0	0	0	0–20	Poor	25	487

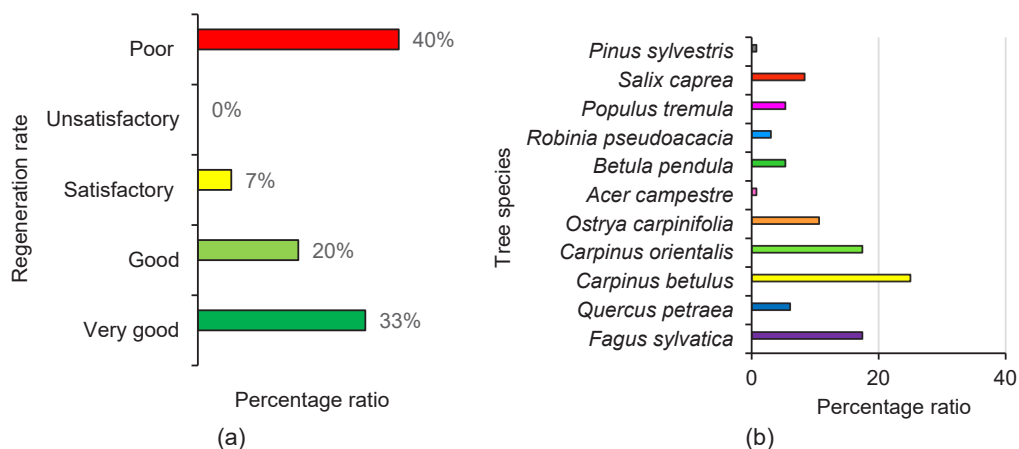


Fig. 2. Natural regeneration in the first transect: (a) assessment according to the applied methodology; (b) regeneration by tree species.

In the sample plots of the second transect, regeneration was more successful, with 87 % of the plots classified as having good or very good regeneration. No plots with absent regeneration were recorded, while only 7 % showed poor regeneration.

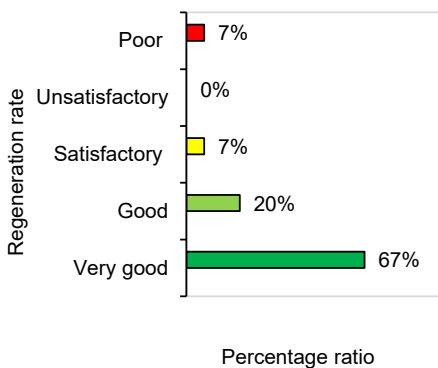
The most commonly occurring tree species were manna ash (*Fraxinus ornus* L.), oriental hornbeam, sessile oak (*Quercus petraea* Matt.), and Austrian black pine.

The results are presented in Table 3 and Figure 3.

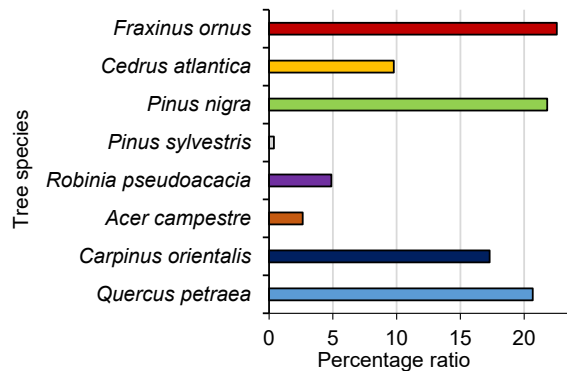
Table 3. Assessment of natural regeneration in the second transect.

Sample plot	Seedling regeneration			Total shoots, pc·ha ⁻¹	Canopy cover, %	Regeneration estimation	Slope, °	Elevation, m a.s.l.
	Shade-tolerant	Helio-philous	Total, shade-tolerant + heliophilous					
1	0	16,960	16,960	0	61–80	Good	26	657
2	0	11,307	11,307	7,538	61–80	Good	26	649
3	0	1884	1,884	0	0–20	Poor	24	638
4	0	11,307	11,307	18,844	81–100	Very good	21	620
5	11,307	18,844	30,151	0	81–100	Very good	24	594
6	30,151	3769	33,920	0	81–100	Very good	26	532

Sample plot	Seedling regeneration			Total shoots, pc·ha ⁻¹	Canopy cover, %	Regeneration estimation	Slope, °	Elevation, m a.s.l.
	Shade-tolerant	Helio-philous	Total, shade-tolerant + heliophilous					
7	0	41,458	41,458	0	81–100	Very good	26	567
8	0	3,769	3,769	5,653	41–60	Satisfactory	38	534
9	0	13,191	13,191	24,498	81–100	Very good	32	514
10	0	13,191	13,191	0	61–80	Good	26	502
11	0	45,227	45,227	0	81–100	Very good	30	482
12	0	47,111	47,111	24,498	81–100	Very good	26	464
13	32,036	30,151	62,187	0	81–100	Very good	22	446
14	0	39,573	39,573	0	81–100	Very good	30	424
15	13,191	35,804	48,996	0	81–100	Very good	29	389



(a)



(b)

Fig. 3. Natural regeneration in the first transect: (a) assessment according to the applied methodology. (b) regeneration by tree species.

The results from the third transect indicated that 90 % of the sample plots exhibited good or very good regeneration, while 10 % showed poor regeneration. The main present tree species were manna

ash, oriental hornbeam, European hornbeam, and sessile oak.

The results from the third transect are presented in Table 4 and Figure 4.

Table 4. Assessment of natural regeneration in the third transect.

Sample plot	Seedling regeneration			Total shoots, pc·ha ⁻¹	Canopy cover, %	Regeneration estimation	Slope, °	Elevation, m a.s.l.
	Shade-tolerant	Helio-philous	Total, shade-tolerant + heliophilous					
1	0	3,769	3,769	28,267	81–100	Very good	22	839
2	3,769	1,884	5,653	18,844	81–100	Very good	24	821
3	28,267	3,769	32,036	11,307	81–100	Very good	22	795
4	0	16,960	16,960	0	61–80	Good	8	772
5	0	1,884	1,884	0	0–20	Poor	10	734
6	15,076	22,613	37,689	0	81–100	Very good	30	672

Sample plot	Seedling regeneration			Total shoots, pc·ha ⁻¹	Canopy cover, %	Regeneration estimation	Slope, °	Elevation, m a.s.l.
	Shade-tolerant	Helio-philous	Total, shade-tolerant + heliophilous					
7	11,307	26,382	37,689	0	81–100	Very good	30	593
8	30,151	16,960	47,111	0	81–100	Very good	31	533
9	52,764	39,573	92,338	0	81–100	Very good	32	489
10	0	20,729	20,729	13,191	81–100	Very good	6	455

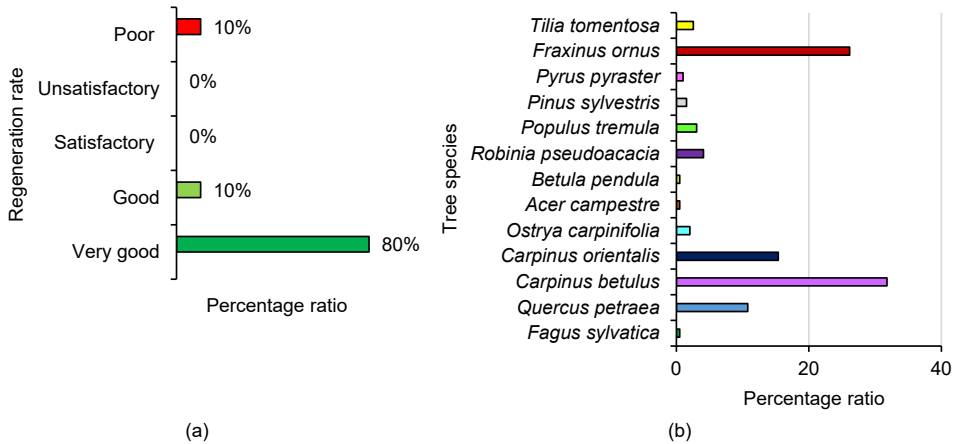


Fig. 4. Natural regeneration in the first transect: (a) assessment according to the applied methodology. (b) regeneration by tree species.

When selecting the transect routes, the aim was to cover different slope aspects. In this way, the available variety of site conditions was included, allowing for a relatively objective overall assessment of regeneration across the burned area. The

elevation difference within the fire-damaged territory exceeds 400 m a.s.l. (ranging from 389 to 869 m a.s.l.). No clear correlation was identified between altitude and the degree of regeneration (Fig. 5).

In the sample plots with a northern aspect

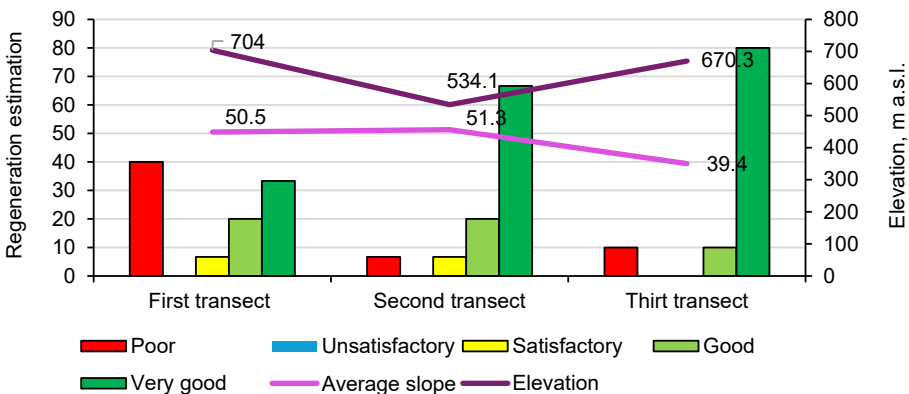


Fig. 5. Total post-disturbance regeneration by average slope and average elevation.

(first transect), higher presence of common beech, a representative of shade-tolerant species, was observed compared with the other two transects (Fig. 6).

The tree vegetation is predominantly represented by light-demanding pioneer species, generally considered to be of lower silvicultural value (Fig. 7).

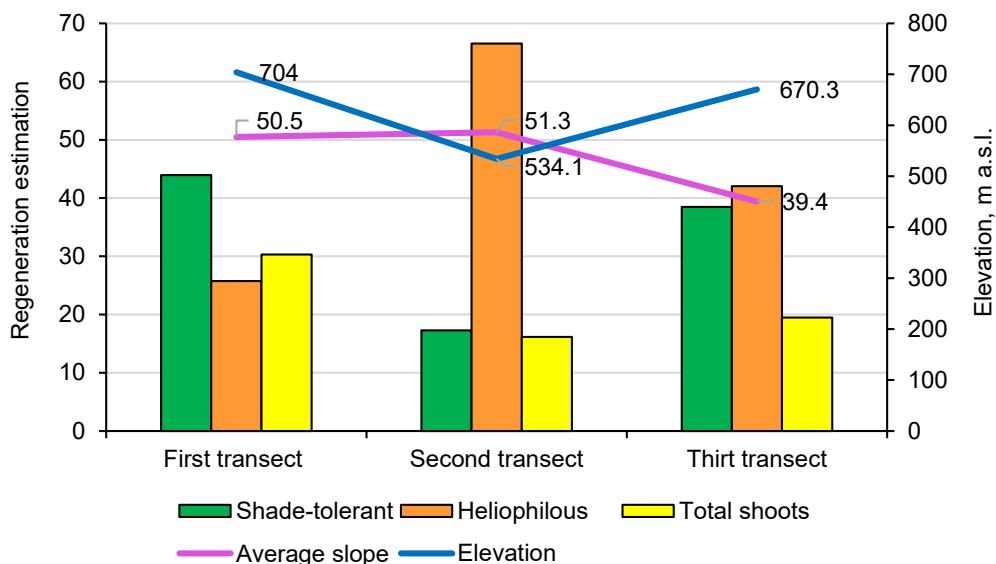


Fig. 6. Total post-disturbance regeneration undergrowth type, average slope, and average elevation.

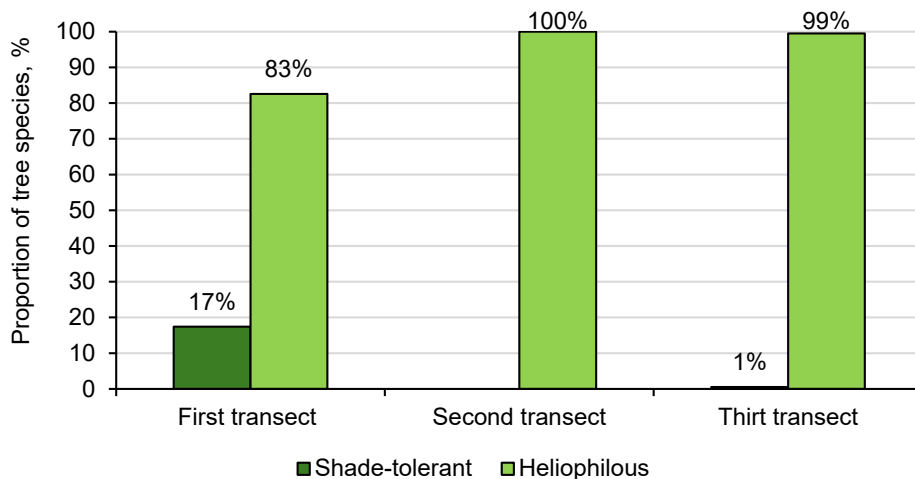


Fig. 7. Proportion of shade-tolerant and light-demanding species by aspect in the three transects.

Discussion

Extreme forest fires increasingly threaten the resilience of forest ecosystems and their capacity to maintain ecological, social, and economic services (EASAC 2025). A key determinant of resilience is natural regeneration. However, following high-severity disturbances, this process is often limited due to altered soil properties, absence of seed sources, and unfavourable climatic conditions, such as rising temperatures and prolonged droughts (Kostov 2024). On steep slopes, the risk of further degradation is particularly high, necessitating anti-erosion measures (Petkova and Tashev 2003, Tashev and Ivanova 2020).

The 2017 wildfire near Kresna illustrates that post-fire recovery in large and complex terrains cannot rely solely on natural processes. In such cases, afforestation has proved essential for controlling erosion and initiating ecosystem restoration (GeoPolymorphic Cloud 2017).

The findings from Dabovo, 15 years after the fire, confirm that natural regeneration is spatially uneven and relatively slow. Compared with earlier regeneration data for common beech in the same forest vegetation zone (Nedelin 1988), recovery appears delayed, with substantial areas still lacking young stands. Although regeneration is more successful on southern and south-eastern slopes, large portions of the burned landscape remain poorly regenerated or entirely devoid of trees.

Similar patterns have been reported in other parts of southern Europe. A study in central Spain found that post-fire Mediterranean shrublands have limited natural recovery potential, particularly where soils are degraded; species from the Genisteae tribe dominate the successional process and stabilise the site (Ugarte et al. 2021).

Furthermore, research from southern Spain demonstrates that high fire recurrence and intensity strongly affect vegetation structure and diversity, often resulting in shrub-dominated rather than forested landscapes (Ortega et al. 2025).

The dominance of pioneer, light-demanding species in Dabovo aligns with typical post-disturbance successional trajectories (Cerioni et al. 2024). Although these species provide valuable ecological functions, their prevalence indicates slow compositional recovery. Shade-tolerant species, such as common beech, are largely confined to cooler, north-facing slopes, highlighting the decisive role of site conditions in shaping regeneration pathways. This pattern reflects a typical successional sequence, where pioneer species first establish structural dominance, gradually facilitating the establishment of more shade-tolerant and longer-lived species. Although some species have lower economic value, they play a critical ecological role in soil stabilisation, biodiversity support, and the provision of habitats for fauna.

Overall, the results suggest that in severely affected areas, particularly on steep and eroded slopes, natural regeneration should be complemented by artificial interventions. Targeted reforestation with local, resilient species, combined with timely silvicultural treatments, can enhance ecosystem resilience and reduce the risk of future high-severity fires (Popov et al. 2016, 2018).

Conclusions

Fifteen years after the Dabovo fire, natural regeneration is spatially uneven, with southern and south-eastern slopes showing the best recovery, whereas north-east-

ern aspects remain poorly regenerated.

Regeneration is dominated by light-demanding pioneer species, while shade-tolerant species are restricted to cooler, north-facing slopes.

Recovery remains slow and incomplete, with substantial areas still lacking regeneration, indicating risk of long-term degradation.

Artificial regeneration should be prioritised on steep, eroded slopes and sun-exposed sites with high fire risk, using local, resilient species.

Long-term monitoring is essential to guide forest management and provide the ecological and functional restoration of post-fire landscapes.

Acknowledgments

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Soil carbon and nitrogen stocks after thinning in Norway spruce plantations in the Central Balkan Mountains, Bulgaria

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Thematic field: Forest policy and key challenges

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Abstract

This study aimed to evaluate how different thinning intensities influence the accumulation of organic carbon and nitrogen in soils. We analysed key soil parameters under varying thinning regimes in Norway spruce (*Picea abies* (L.) H.Karst.) stands, compared with unthinned controls, across six state forestry management units in the Central Balkan Mountains, Bulgaria. On the territory of each management unit, soil samples were taken from three types of plots: unmanaged controls (no silvicultural intervention for over 20 years); light thinning (10–15 %, within the past 10 years); and moderate thinning (20–25 %, within the past 10 years). Soils were examined in depth layers of 0–10, 10–20 and 10–30 cm. We measured bulk density, rock content, soil acidity, and soil organic carbon and total nitrogen stocks. Results indicate that low-intensity thinning (10–15 %) significantly alters soil organic carbon. These findings highlight the need for proactive management strategies to guide thinning practices in Bulgarian forests, with particular attention to soil organic carbon and total nitrogen dynamics.

Key words: forest management, mountain ecosystems, organic matter, soil depth, sustainable management, thinning intensity.

Introduction

Forests are essential regulators in the global biogeochemical cycles of carbon (C) and nitrogen (N), acting as dynamic C sinks through biomass, litter, and soil (Lal 2005, Pan et al. 2011). Forest soils, in

particular, store approximately two-thirds of ecosystem C, and they are crucial for N cycling, soil buffering, and water retention (Batjes 1996, Galloway et al. 2008). The role of soil as a stabilising factor in ecosystem functioning is especially important in mountain regions, where climatic and

topographic variability interact strongly with land use and forest structure (Zhiyanski et al. 2016, Kirova 2024).

In Bulgaria, Norway spruce (*Picea abies* (L.) H.Karst.) is widespread in the montane and subalpine zones of the Central Balkan Mountains, occurring in both natural forests and plantations (Alexandrov and Dimitrov 2005, Panayotov et al. 2011). These coniferous plantations, established during large-scale reforestation campaigns, now play a dual role – providing timber and regulating key ecological processes, such as water retention, erosion control, and C sequestration (Zhiyanski et al. 2016, Kirova 2018). However, many Norway spruce stands are currently at or near maturity, making them vulnerable to wind damage, pests, and drought – especially when artificially structured and densely stocked (Panayotov et al. 2015, Menichetti et al. 2017, Marinov et al. 2022).

Forest thinning is widely applied in these systems to improve tree stability and resilience, reduce competition, and maintain ecosystem functions. However, thinning affects the microclimate and soil surface through increased radiation, altered water dynamics, and changes in litterfall, with direct consequences for soil C and N dynamics (Jandl et al. 2007, Zhang et al. 2024). Studies have shown that thinning can decrease C inputs to the soil due to reduced root exudation and organic debris, while simultaneously enhancing decomposition and mineralisation (Zhang et al. 2018, Stoeva and Kirova 2021). However, when conducted at low intensity and with attention to site-specific conditions, thinning does not necessarily reduce soil organic carbon stocks (SOC_{stocks}) and may even improve N availability in upper horizons (Menichetti et al. 2017, Kirova 2024). Thinning can stimulate microbial

processing and nutrient cycling, leading to greater incorporation of stable organic matter over time, which could bring the system to post-disturbance recovery of soil organic carbon (SOC), when below-ground C inputs and nutrient cycling processes are enhanced (Zhang et al. 2023). In addition, some studies have indicated that minimal interventions trigger net losses, but moderate interventions promote recovery, especially if logging residues are retained (Zhang et al. 2022, Erkan et al. 2023).

Despite the importance of these findings, regional studies in Bulgaria remain sparse. Recent work in the western Stara Planina indicates that thinning intensities of around 25 % in Norway spruce plantations do not significantly alter bulk density (BD), pH, or coarse fraction content (CF), although slight reductions in SOC and significant increases in total nitrogen (TN) were observed in managed plots compared to control plots without management (Kirova 2024). The type and intensity of disturbance – whether silvicultural or natural, such as ice storms – also affect nutrient cycling and C stocks over decadal timescales (Menichetti et al. 2017, Kirova 2024).

This study builds on these findings by evaluating the effects of various thinning intensities on key soil parameters in Norway spruce plantations in the Central Balkan Mountains. The investigation focuses on SOC_{stock} and total nitrogen stock (TN_{stock}), bulk density, soil reaction (pH), CF, and soil moisture (W), measured across three soil depth layers (0–10, 10–20, and 20–30 cm). The aim of the study was to evaluate how thinning intensity influences the accumulation of SOC and TN, thereby providing a basis for sustainable forest management in the studied type and similar coniferous plantations.

Materials and Methods

Research area

This study was conducted in 2022 on the territory of six state forestry management units (SFMU) located in the central part of the Balkan Mountains (Stara Planina), Bulgaria, specifically on the northern slopes of the range: Etropole, Teteven, Troyan, Apriltsi, Rositsa, and Gabrovo (Fig. 1).

The research area is located at altitudes ranging from 1000 to 1300 m a.s.l. The soils in the studied plots are classified as Cambisols (IUSS Working Group WRB 2015). The focus of the study was on managed Norway spruce plantations, subjected to thinning at different intensities.

Experimental design

In each SFMU, three sample plots were established in Norway spruce plantations (Table 1) with different forest thinning intensities:

- M0 – Control plots: unmanaged stands with no silvicultural intervention in the past 20+ years;
- M10 – Light-intensity thinning: plots thinned at 10–15 % within the past 10 years;
- M20 – Moderate-intensity thinning: plots thinned at 20–25 % within the past 10 years.

The information on thinning intensity was obtained from the official forest management plans and refers to the reduction in stand volume of the studied plantations.

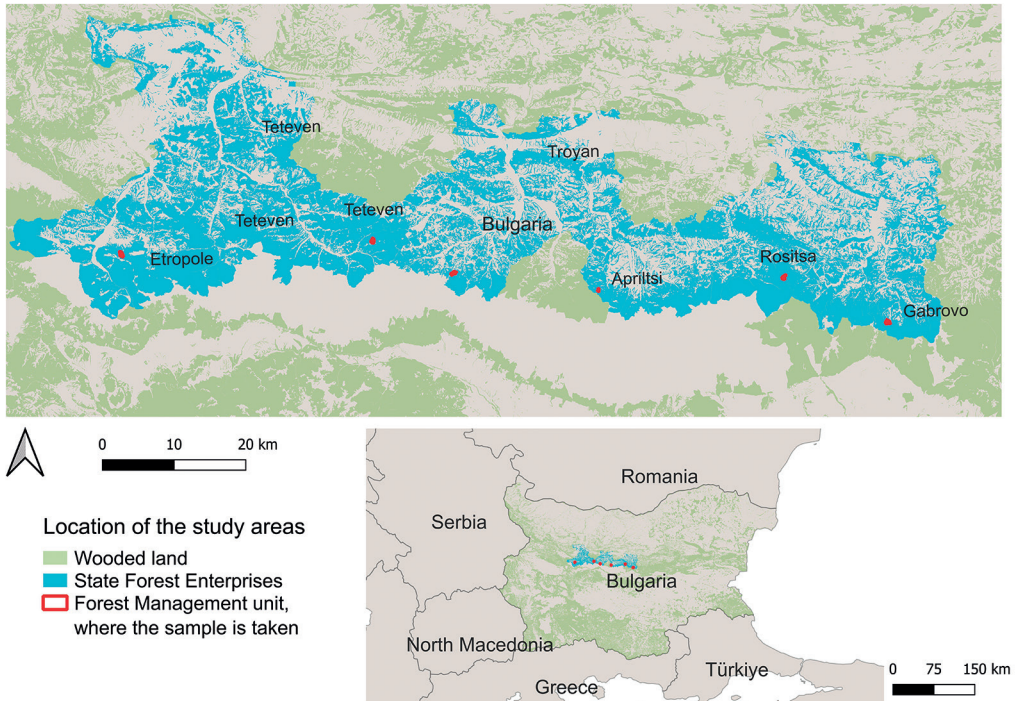


Fig. 1. Location of the study area in the Central Balkan Mountains, Bulgaria.

Table 1. Characteristics of sample plots.

State forestry enterprise sample site	Soil type, IUSS 2015	Aspect of the slope	Altitude, m a.s.l.	Mean slope, °	Stand age, years	Year of thinning
Etropole						
M0	Cambisols	NE	1200	27	50	-
M10		NE	1200	27	50	2013
M20		NE	1200	27	50	2013
Teteven						
M0	Cambisols	NW	1200	26	65	-
M10		NW	1200	26	65	2015
M20		NW	1200	26	65	2015
Troyan						
M0	Cambisols	N	1300	21	45	-
M10		N	1300	21	45	2015
M20		N	1250	14	60	2010
Apriltsi						
M0	Cambisols	N	1300	24	55	-
M10		NW	1250	29	55	2014
M20		N	1250	24	55	2013
Rositsa						
M0	Cambisols	NW	1100	26	50	-
M10		NW	1100	26	50	2013
M20		NW	1100	26	50	2013
Gabrovo						
M0	Cambisols	N	1000	26	50	-
M10		N	1000	26	50	2014
M20		N	1000	26	50	2014

Note: IUSS 2015 = IUSS Working Group WRB (2015).

Each sample plot covered 0.1 ha (1000 m²). Soil samples were collected, after removing the litter layer, using a stainless-steel auger from three depth layers: 0–10 cm, 10–20 cm, and 20–30 cm. For each depth, three replicate soil samples were randomly collected within each 0.1 ha plot. The random placement of sampling points was designed to capture microrelief heterogeneity (e.g., small slope variations, ground vegetation patches) and reduce the influence of within-plot variability on the results (FAO 2006).

Laboratory methods and approaches

Following ISO 11465:1993, soil samples were oven-dried at 105 °C for 5 h to determine soil moisture (W) and bulk density (BD).

BD and W were calculated using equations (1) and (2):

$$BD = \frac{WD}{VC}, \quad (1)$$

where:

- BD is the soil bulk density, g·cm⁻³;
- WD is the dry weight of the soil sample, g;

- VC is the volume of the sampling cylinder, cm³.

$$W = \frac{WW - WD}{WD} \cdot 100, \quad (2)$$

where:

- W is the soil moisture content, %;
- WW is the wet weight of the sample, g;
- WD is the dry weight of the sample, g.

The CF was determined gravimetrically (Donov et al. 1974) following these steps:

1. Approximately 100 g of dried soil was subsampled;
2. Organic matter was removed by washing;
3. The residue was dried and sieved through a 2 mm mesh;
4. Fragments >2 mm were weighed;
5. Calculated according to the following equation (3).

$$CF = \frac{WCF}{WMS} \cdot 100, \quad (3)$$

where:

- CF is the coarse fragment content, %;
- WCF is the weight of coarse fragments, g;
- WMS is the weight of the measured subsample, g.

Soil texture (mechanical composition) was analysed using Kaczynski pipette method, after dispersion with hydrochloric acid (Donov et al. 1974).

Chemical analyses were performed on sieved samples (<2 mm). Soil pH was measured using a HANNA HI2211 pH/ORP meter in a 1:5 soil-to-water suspension, following ISO 10390:1994.

SOC was determined using the Tjurin method via oxidation with a K₂Cr₂O₇-H₂SO₄ mixture at 160 °C, catalysed by pumice and Ag₂SO₄ (Donov et al. 1974). Titration was carried out with 0.2N Mohr's salt ((NH₄)₂SO₄·FeSO₄·6H₂O) with phenylanthranilic acid as an indicator.

TN was determined by the Kjeldahl method, in accordance with ISO 11261:1995 (1995). The C:N ratio was calculated from the measured SOC and TN values.

SOC_{stock} was estimated by equation (4), according to the IPCC Good Practice Guidance for Land Use, Land-Use Change and Forestry (IPCC 2003). TN_{stock} was calculated using the same approach, following Ellert and Bettany (1995):

$$\text{SOC}_{\text{stock}} \text{ or } \text{TN}_{\text{stock}} = C \text{ or } N (\%) \cdot \text{BD} (\text{g} \cdot \text{cm}^{-3}) \cdot d (\text{cm}) \cdot (1 - CF), \quad (4)$$

where:

- C or N is the content of soil organic carbon or total nitrogen, %;
- BD is soil bulk density, g·cm⁻³;
- d is depth of the soil layer, cm;
- CF is the fraction of coarse fragments (>3 mm) expressed as a decimal.

Statistical analyses

Statistical analyses were conducted using the R environment (R Core Team 2024) via the RStudio interface, version 2024.09.0+375 (Posit Software 2024). For each of the six sample plots within the three thinning intensities (M0, M10, M20), the mean values of the three field replicates per plot were calculated and used in subsequent analyses.

Normality of the data was assessed separately for each soil depth (0–10, 10–20, and 20–30 cm) and thinning intensity using the Shapiro–Wilk test.

Two-way ANOVA was performed to evaluate the effects of thinning intensity and soil depth on the main soil properties. When significant differences were detected ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to identify pairwise differences among groups between thinning intensities and depths.

Results

Across all measured parameters, soil depth exerted a strong influence on soil physical and chemical properties.

Depth-related gradients were evident for W, BD, CF, SOC, TN, the C:N ratio, and SOC_{stock} and TN_{stock}. The average values along with standard deviations are presented in Table 2.

Table 2. Soil properties by depth and thinning intensity.

Parameter	Depth, cm	M0	M10	M20
W, %	0–10	24.49 ±8.94	30.02 ±8.40	24.53 ±10.11
	10–20	22.12 ±6.10	23.45 ±5.55	22.57 ±8.74
	20–30	22.19 ±6.91	20.67 ±5.17	22.66 ±9.19
BD, g·cm ⁻³	0–10	0.98 ±0.11	0.96 ±0.16	0.95 ±0.15
	10–20	1.09 ±0.12	1.13 ±0.19	1.11 ±0.11
	20–30	1.18 ±0.10	1.22 ±0.09	1.09 ±0.12
CF, %	0–10	30.96 ±4.83	21.96 ±5.24	26.02 ±4.43
	10–20	24.64 ±7.32	22.89 ±6.85	23.84 ±3.51
	20–30	29.33 ±5.09	25.59 ±5.07	21.63 ±6.35
pH	0–10	3.59 ±0.37	3.71 ±0.33	3.63 ±0.31
	10–20	3.73 ±0.39	3.70 ±0.24	3.57 ±0.28
	20–30	3.62 ±0.41	3.78 ±0.28	3.64 ±0.30
SOC, %	0–10	6.14 ±1.59	4.96 ±1.58	5.61 ±1.14
	10–20	4.38 ±1.08	3.45 ±0.77	4.01 ±1.04
	20–30	3.16 ±1.00	1.95 ±0.19	2.90 ±0.85
TN, %	0–10	0.35 ±0.13	0.31 ±0.10	0.33 ±0.13
	10–20	0.28 ±0.10	0.23 ±0.05	0.26 ±0.09
	20–30	0.23 ±0.08	0.17 ±0.04	0.21 ±0.07
C:N	0–10	19.18 ± 6.92	16.30 ± 5.00	14.41 ± 3.06
	10–20	16.79 ± 6.97	15.38 ± 3.53	12.27 ± 3.97
	20–30	18.55 ± 6.36	15.73 ± 2.76	13.93 ± 1.10
SOC _{stock}	0–10	40.48 ± 8.07	35.24 ± 7.22	25.94 ± 7.04
	10–20	35.60 ± 6.85	29.03 ± 2.74	17.69 ± 2.35
	20–30	38.33 ± 3.24	33.22 ± 5.97	24.55 ± 7.36
TN _{stock}	0–10	2.30 ± 0.82	2.29 ± 0.73	1.86 ± 0.62
	10–20	2.29 ± 0.63	1.94 ± 0.32	1.53 ± 0.38
	20–30	2.25 ± 0.70	2.18 ± 0.60	1.79 ± 0.61
Clay, %	0–10	23.61 ±8.87	31.10 ±7.96	31.84 ±8.80
	10–20	34.44 ±11.09	32.66 ±8.74	33.17 ±6.95
	20–30	33.63 ±8.97	42.84 ±16.36	32.09 ±6.69
Sand, %	0–10	76.39 ±8.87	68.90 ±7.96	68.16 ±8.80
	10–20	65.56 ±11.09	67.34 ±8.74	66.83 ±6.95
	20–30	66.37 ±8.97	57.16 ±16.36	67.91 ±6.69

Note: M0 = control (unthinned), M10 = light-intensity thinning (10–15 %), M20 = moderate-intensity thinning (20–25 %). Bulk density (BD), soil moisture (W), coarse fragments (CF), organic carbon (SOC), total nitrogen (TN), clay, and sand as percentages. Depth intervals refer to the top 0–10, 10–20, and 20–30 cm of the mineral soil. Columns 3–5 represent mean ±standard error.

W was generally highest in the 0–10 cm layer, with M10 plots showing the highest mean value (30.02 ± 8.40 %), followed by M0 (24.49 ± 8.94 %) and M20 (24.53 ± 10.11 %). At 20–30 cm, W values decreased, particularly in M10 (20.67 ± 5.55 %), whereas M0 and M20 maintained slightly higher levels (Fig. 2).

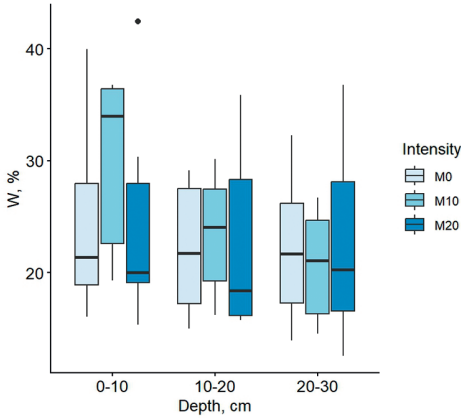


Fig. 2. Distribution of W across all studied depths and treatments.

BD increased with depth (Fig. 3), ranging from ~ 0.95 – 0.98 $\text{g}\cdot\text{cm}^{-3}$ in the surface layer to ~ 1.18 – 1.22 $\text{g}\cdot\text{cm}^{-3}$ at 20–30 cm

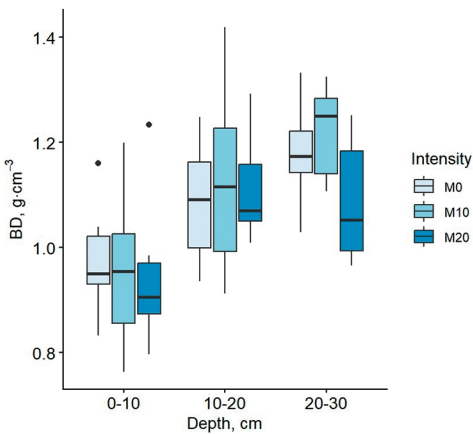


Fig. 3. Distribution of BD across all studied depths and treatments.

(Table 2). The highest BD was observed in M10 plots at 20–30 cm (1.22 ± 0.19 $\text{g}\cdot\text{cm}^{-3}$).

CF was highest in the 0–10 cm layer of M0 (30.96 ± 4.83 %) and lowest in M10 (21.96 ± 5.24 %). With increasing depth, CF tended to decrease in M0 between 0 and 20 cm, but slightly increased again at 20–30 cm. In contrast, CF showed greater fluctuations in M10 and M20 (Fig. 4).

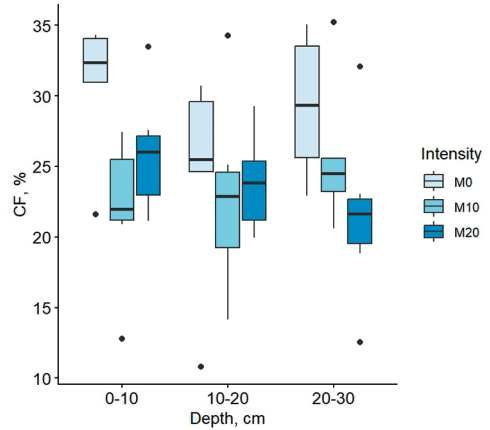


Fig. 4. Distribution of CF across all studied depths and treatments.

Soil pH ranged between 3.57 and 3.78, with slightly higher mean values in deeper layers of M10 (Fig. 5).

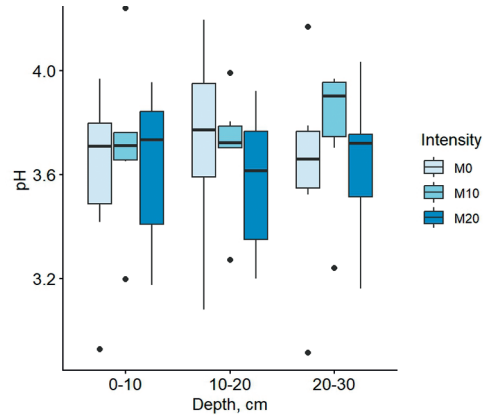


Fig. 5. Distribution of soil pH (in d.H₂O) across all studied depths and treatments.

Soil texture (clay and sand fractions) remained relatively consistent across treatments within each depth layer, but some depth-related variation was observed. Clay content tended to increase with depth in all treatments, with the highest value recorded in M10 at 20–30 cm ($42.84 \pm 16.36\%$). Conversely, sand content decreased slightly with depth in M10, reflecting a finer texture in deeper layers. SOC and TN declined sharply with depth (figs 6A and

7A). In 0–10 cm layer, SOC ranged from $4.96 \pm 1.58\%$ in M10 to $6.14 \pm 1.59\%$ in M0, while TN ranged from $0.31 \pm 0.10\%$ in M10 to $0.35 \pm 0.13\%$ in M0. At 20–30 cm, SOC and TN declined to $1.95 \pm 0.77\%$ and $0.17 \pm 0.05\%$, respectively.

SOC_{stock} was highest in M0 plots in the 0–10 cm layer ($40.48 \pm 8.07 \text{ Mg C} \cdot \text{ha}^{-1}$) and decreased with both depth and thinning intensity (Fig. 6B), reaching the lowest value in M10 plots at 20–30 cm (17.69

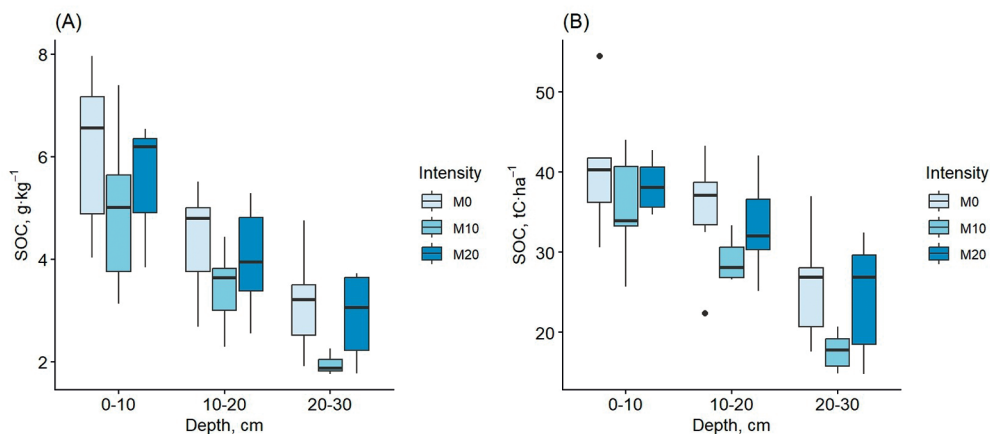


Fig. 6. Distribution of SOC across all studied depths and intensities: (A) SOC; (B) SOC_{stock}.

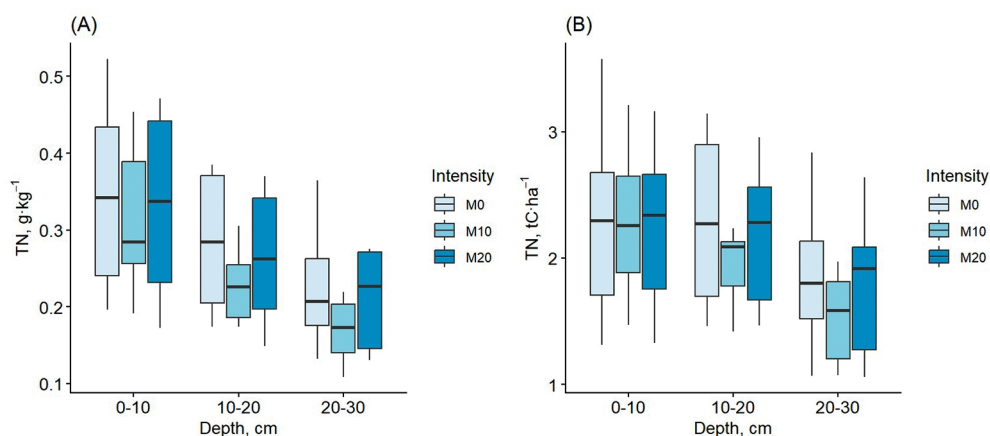


Fig. 7. Distribution of TN across all studied depths and intensities: (A) TN; (B) TN_{stock}.

$\pm 2.74 \text{ Mg C} \cdot \text{ha}^{-1}$).

TN_{stock} changes followed the trend observed in SOC_{stock} (Fig. 7B), declining with depth and showing slightly lower values in thinned plots.

The C:N ratio decreased from ~16–19 in topsoil to ~12–15 in deeper layers, with consistently higher values in M0 plots (Fig. 8).

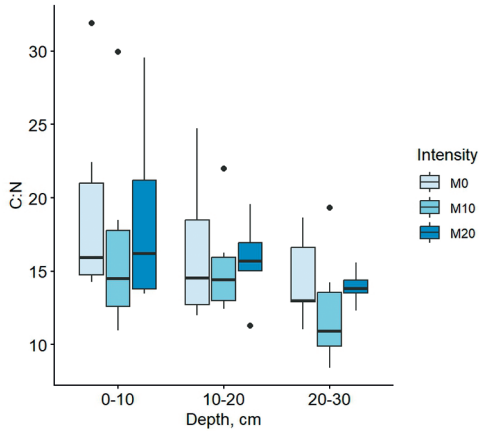


Fig. 8. Distribution of the C:N ratio across all studied depths and intensities.

Two-way ANOVA confirmed that soil depth represents a significant factor affecting most of the measured parameters, while thinning intensity had more limited but still detectable influence on selected chemical properties.

For SOC and SOC_{stock}, both soil depth

and thinning intensity had significant effects (Table 3). Both parameters vary significantly with depth with $F = 34.00$ and $p < 0.001$ for SOC, and $F = 32.17$ and $p < 0.001$ for SOC_{stock}. The thinning intensity's effect on SOC and SOC_{stock} is statistically significant – $F = 5.08$ and $p < 0.01$ for SOC, and $F = 5.82$ and $p < 0.01$ for SOC_{stock}.

Tukey's HSD test indicated that SOC decreased significantly from 0–10 cm to deeper layers, with all depth categories differing from each other (Table 3). Regarding thinning, M10 plots had significantly lower SOC and SOC_{stock} than M0 plots, while M20 showed intermediate values that did not differ significantly from either M0 or M10.

For TN and TN_{stock}, soil depth had a significant effect, with the highest N concentrations observed in the topsoil. There is no significant difference in the means of TN and TN_{stock} between the studied thinning intensities within a single soil layer. According to the two-way ANOVA, thinning intensity did not have a significant influence on either TN or TN_{stock} (Table 3).

Overall, the analysis indicated that thinning intensity had a detectable effect primarily on SOC and SOC_{stock}, while its influence on TN was negligible compared to the dominant effect of soil depth.

Table 3. Two-way ANOVA results (F and p values) and Tukey HSD pairwise comparisons across soil depths and thinning intensities.

Parameter	ANOVA-Depth		ANOVA-Intensity		Tukey's test		
	p -value	F -value	p -value	F -value	M0	M10	M20
SOC	<0.01	34.00	<0.01	5.08	a	b	ab
TN	<0.01	9.40	0.29	1.37	a	a	a
C:N	0.02	4.54	0.49	0.73	a	a	a
SOC _{stock}	<0.01	32.17	<0.01	5.82	a	b	ab
TN _{stock}	0.02	4.14	0.50	0.70	a	a	a

Discussion

Our study in Norway spruce plantations of the Central Balkan Mountains revealed that soil depth was a primary driver of variation in physical and chemical soil properties, while thinning intensity had more limited and parameter-specific effects.

Soil properties in this study followed typical depth-related patterns for mountain Norway spruce plantations, with higher *W* and lower *BD* in surface layers and more compact, less moist soils at depth, regardless of thinning treatment. Slightly higher surface *W* in M10 may reflect localised organic matter accumulation, although part of the variation may also be related to short-term weather conditions at the time of sampling, while increased *BD* at depth likely results from natural compaction rather than thinning (Lal 2005, Vesterdal et al. 2013). *CF* and *pH* varied modestly, but differences were inconsistent and likely site-specific, consistent with reports that these attributes are primarily controlled by parent material and long-term pedogenesis (Donov 1973). The absence of statistically significant treatment effects suggests that, in the short term, thinning does not markedly alter these relatively stable soil parameters. Our observations support previous findings that canopy disturbance influences dynamic soil properties, such as $\text{SOC}_{\text{stock}}$ and TN_{stock} , to a greater extent than the stable physical attributes (Bai et al. 2017). The vertical stratification of *SOC*, *TN*, and *C:N* ratio – characterised by higher values in the topsoil and declining with depth – is consistent with patterns reported for temperate and montane forest ecosystems (Jandl et al. 2007). This decline is largely attributable to the decreasing organic matter inputs from litterfall, fine root turnover, and microbial activity with increasing soil depth (Vesterdal et

al. 2013).

Thinning influenced *SOC* and $\text{SOC}_{\text{stock}}$ in the 0–10 cm layer, with the 10–15 % thinning intensity (M10) showing lower values compared to unthinned controls (M0), while the 20–25 % thinning intensity (M20) showing intermediate results. This non-linear response suggests that thinning intensity can influence the balance between *SOC* losses and inputs, with potentially important implications for sustainable management of Norway spruce plantations in the Central Balkan Mountains.

A possible explanation could be connected with the mechanical differences between these intensity levels. Light thinning typically induces small canopy openings that increase soil temperature and aeration, enhancing microbial decomposition and accelerating organic matter mineralisation (Xu et al. 2022). However, the canopy disturbance may be insufficient to stimulate significant understorey growth or root turnover, resulting in lower organic matter inputs from litter and rhizodeposition (Hoover 2011). This imbalance can lead to net *SOC* losses, particularly in surface layers where microbial activity is highest (Vesterdal et al. 1995).

In contrast, moderate thinning creates larger canopy gaps, which can stimulate understorey vegetation, increase fine-root biomass, and promote higher litterfall rates (Livne-Luzon et al. 2025). Enhanced belowground carbon inputs, coupled with improved nutrient cycling, can compensate for the initial disturbance-related *SOC* losses and facilitate recovery over time (Gao et al. 2023). Additionally, reduced stand density may alleviate competition for soil resources, allowing residual trees to increase their root allocation and contributing further to soil organic matter accumulation (He et al. 2025). Retention of slash and forest floor material

can enhance SOC stabilisation during the post-thinning recovery phase (Qu et al. 2025).

It is important to recognize that thinning effects on SOC are strongly influenced by site conditions, soil depth, and time since intervention (Xu et al. 2024). Forest floor carbon is generally more sensitive to thinning than mineral soil carbon, and deeper soil layers may respond differently from the topsoil (Gross et al. 2018). Long-term monitoring is thus essential to capture the trajectory of SOC dynamics beyond the short-to-medium term.

In contrast, TN and TN_{stock} were largely unaffected by thinning intensity, suggesting that nitrogen cycling processes in these Norway spruce plantations are more stable. As it was already mentioned, thinning alters microclimate and can stimulate mineralisation and nitrification (affecting N forms), but retention pathways (e.g., microbial immobilisation, canopy/forest-floor feedbacks, and residue retention) tend to buffer net TN_{stock} at the scale of 0–30 cm over short–medium terms, unless disturbance is severe or residues are removed (Formánek and Vránová 2003, Bai et al. 2017). This buffering could be due to stable inputs from symbiotic mycorrhiza and slow mineralisation rates characteristic of acid, organic-rich mountain soils (Ekblad et al. 2013, Kirova 2018). The finding that TN and TN_{stock} were largely unaffected by thinning intensity is consistent with broader evidence: light–moderate thinning typically shifts N cycling processes (availability, transformations) but does not necessarily translate into rapid changes in total soil N pools, which are slower to respond and strongly conditioned by site factors and time since treatment (Xu et al. 2024).

Our findings are important for sustainable forest management in Bulgaria, where Norway spruce plantations –

though not native to all elevations – play a critical role in timber production, erosion prevention, and water regulation (Alexandrov and Dimitrov 2005, Panayotov et al. 2011). The minimal effects of thinning on most soil properties suggest that carefully planned low-to-moderate thinning can be compatible with soil conservation goals, provided that surface organic matter is preserved and post-thinning regeneration is promoted.

However, the observed SOC reductions under low-intensity thinning highlight the need for long-term monitoring to detect potential cumulative impacts. Climate change scenarios project increased drought frequency and temperature rise in the Balkan region (IPCC 2021), which could exacerbate SOC losses and alter TN dynamics. Future studies should integrate microbial community analyses, litter quality assessments, and fine root productivity to link observed changes in soil parameters to belowground biological processes (Prescott 2010, Schmidt et al. 2011).

Overall, this research emphasizes that depth-stratified sampling and treatment-specific statistical analysis can detect subtle but ecologically meaningful changes. Such approaches should be standard practice when evaluating forest management impacts on soil, especially in ecologically sensitive montane regions. In the context of Norway spruce plantations in the Central Balkan Mountains, these results suggest that moderate thinning (≈ 20 – 25 %) could represent a more sustainable approach for balancing timber production with soil conservation. By optimizing canopy openings to stimulate productive understories and belowground inputs, managers can mitigate SOC losses while maintaining forest productivity and resilience.

Conclusions

Forest management practices, particularly thinning, play a critical role in regulating soil carbon and nitrogen storage in Norway spruce plantations. The results of this study indicate that light thinning (M10) can lead to greater reductions in SOC content and stock, compared to moderate-intensity thinning (M20). The TN content and stocks were largely unaffected. These findings indicate that the relationship between thinning intensity and SOC and TN dynamics is not linear, highlighting the importance of adjusting management practices to site-specific conditions.

Careful consideration of thinning intensity is essential for maintaining soil health, fertility, and long-term productivity. Sustainable forest management in Norway spruce plantations requires balancing timber production objectives with the preservation of soil functions that support ecosystem stability, water regulation, and climate change mitigation.

Long-term monitoring is needed to better understand the cumulative effects of thinning on soil C and N dynamics over time. Additionally, investigating the underlying mechanisms behind the observed differences between thinning intensities – such as changes in microbial activity, litter decomposition rates, and root system dynamics – would provide valuable insights for refining silvicultural guidelines.

Overall, this analysis offers a more detailed understanding of how thinning intensity influences soil properties, reinforcing the need to integrate soil conservation objectives into forest management planning.

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High-rank syntaxa of riparian shrubland and forest vegetation in Bulgaria

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Thematic field: Ecosystem biodiversity, conservation and environmental restoration

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Abstract

Riparian shrub and forest vegetation is widespread across Bulgaria, but its syntaxonomic diversity has not been fully studied, which was the main aim of our study. All published relevés were compiled, and new data were collected from different parts of the country, e.g. 905 relevés. Hierarchical clustering was used for classification from class to alliance levels. Diagnostic, constant, and dominant species were identified for all established syntaxa. This study provides the first comprehensive investigation into the distribution, syntaxonomic diversity, ecological characteristics, and threats to riparian forests and shrublands in Bulgaria. Riparian vegetation was classified into two classes, four orders, and seven alliances: 1) *Alno glutinosae-Populetea albae* P. Fukarek

et Fabijanić 1968, *Populetales albae* Br.-Bl. ex Tchou 1949, *Platanion orientalis* I. Kárpáti et V. Kárpáti 1961, *Alno-Fraxinetalia excelsioris* Passarge 1968, *Alnion incanae* Pawłowski et al. 1928, *Alno-Quercion roboris* Horvat 1950; and 2) *Salicetea purpureae* Moor 1958, *Salicetalia purpureae* Moor 1958, *Salicion albae* Soó 1951, *Salicion eleagno-daphnoidis* (Moor 1958) Grass 1993, *Salicion triandrae* T. Müller and Görs 1958, *Tamaricetalia ramosissimae* Borza et Boscaiu ex Doltu et al. 1980, *Tamaricion parviflorae* I. Kárpáti and V. Kárpáti 1961. The Mediterranean influence in the country is shown in forests of *Alno-Quercion roboris*, *Platanion orientalis*, and *Tamaricion parviflorae*, found in the southern part of Bulgaria. Alliances *Alnion incanae* and *Salicion eleagno-daphnoidis* occur in the semi-mountainous and mountainous regions, whereas phytocoenoses of the *Salicion albae* alliance are widespread in plains along rivers and water bodies. Alliance *Salicion triandrae* includes shrub communities dominated by *Amorpha fruticosa* L. and *Salix triandra* L. On the other hand, the alliance *Alno-Quercion roboris* is restricted to rivers in northern Bulgaria and the temperate region. The species composition of the studied vegetation was influenced by continentality, which affects climate patterns, such as light and temperature regimes. Additionally, moisture availability and altitude also played a significant role in shaping the studied vegetation.

Key words: Braun-Blanquet approach, classification, habitats, wetlands.

Introduction

Riparian zones represent unique and diverse transitional areas or ecotones that bridge land and water. These include places like riverbanks, stream banks, floodplains, wetlands, and littoral zones, both lotic and lentic, all of which offer more varied habitat conditions than the surrounding areas (Capon 2020). Riparian forests are complex and variable ecosystems that edge between terrestrial and aquatic systems, characterised by steep environmental gradients and supporting high levels of local biodiversity (Naiman et al. 1998, Tolkkinen et al. 2020). Recognised as climatic refugia for wildlife, and particularly for plant biodiversity, riparian areas are recommended as significant conservation areas for mitigating the impacts of climate change (Zhang et al. 2023).

Riparian forests can provide a variety of ecosystem services. Riis et al. (2020) summarize the ecosystem services that riparian vegetation provides, indicating the

diverse benefits these services offer for human well-being, such as provisioning services, regulating and maintenance services, cultural services. Dinca et al. (2025) recognised biodiversity support, carbon sequestration, water quality regulation, slope stability, pollution mitigation and socio-cultural benefits as core ecosystem services delivered by riparian forests.

Nevertheless, riparian zones are sensitive and vulnerable ecosystems, often modified and disturbed by human activities. Climate change, civil infrastructure, alien species, agricultural practices and pollutant inputs impact and degrade their buffer capacity, disrupt vital ecosystem functions, and diminish the provision of valuable ecosystem services (Capon et al. 2013, Arif et al. 2021, Pandey et al. 2022).

The syntaxonomy of alluvial forests and scrub is related to *Alno glutinosae-Populetea albae* P. Fukarek et Fabijanić 1968 and *Salicetea purpureae* Moor 1958 of the azonal vegetation classes, en-

compassing the European riparian forest galleries and willow and tamarisk scrub (Mucina et al. 2016). Both vegetation classes are present throughout Europe and cover the whole territory of the Balkan Peninsula (Preislerová et al. 2022).

According to the checklists of the Bulgarian syntaxa, alluvial riparian galleries of *Alno glutinosae-Populetea albae* class are represented by the alliances *Populion albae* Br.-Bl. ex Tchou 1949, *Platanion orientalis* I. Kárpáti et V. Kárpáti 1961, *Alnion incanae* Pawłowski et al. 1928, *Fraxino-Quercion roboris* Passarge 1968, *Alno-Quercion roboris* Horvat 1950, whereas willow and tamarisk scrub of *Salicetea purpureae* class is represented by the alliances *Salicion eleagno-daphnoidis* (Moor 1958) Grass 1993, *Salicion albae* Soó 1951, *Salicion triandrae* T. Müller and Görs 1958, *Tamaricion parviflorae* I. Kárpáti and V. Kárpáti 1961, *Artemisio scopariae-Tamaricion ramosissimae* Simon and Dihoru 1963 (Tzonev et al. 2009, Apostolova 2023). Up to now, *Alno glutinosae-Populetea albae* class has been researched in Bulgaria by Pavlov and Dimitrov (2002), Dimitrov et al. (2005), Tzonev (2009b), Tzonev et al. (2009), Pedashenko et al. (2015), Douda et al. (2016), Petrova and Tzonev (2020); Alexandrova et al. (2020), Georgiev (2021), Nikolov and Dimitrov (2023), whereas *Salicetea purpureae* class has been investigated by Tzonev (2009a,b), Tzonev et al. (2009, 2019), Kalníková et al. (2014, 2021), Grigorov et al. (2023a, 2023b), Nikolov and Dimitrov (2023). Some of the riparian zones are listed under the Red Data Book of the Republic of Bulgaria, vol. 3, and under the Habitat Directive as being threatened habitat types: 31F9 Riverside Tamarisk (*Tamarix* spp.) stands [HD 92/43: 92D0 Southern riparian galleries and thickets (*Nerio-Tamaricetea* and *Se-*

curinegion tinctoriae] – category Endangered [EN], 01G1 Riverside poplar-willow forests [HD 92/43: 91E0 *Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*)] – category Endangered [EN], 02G1 Flooded forests of Common alder (*Alnus glutinosa*) [HD 92/43: 91E0 *] – category Endangered [EN], 03G1 Mountain Grey alder (*Alnus incana*) galleries [HD 92/43: 91E0 *] – category Vulnerable [VU], 04G1 Riverside woodlands of Alder (*Alnus* spp.) and Common ash (*Fraxinus excelsior*) [HD 92/43: 91E0 *] – category Vulnerable [VU], 05G1 Riparian and lowland mixed woodlands and longozes [HD 92/43: 91F0 Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia*, along the great rivers (*Ulmion minoris*)] – category Critically endangered [CR], 06G1 Willow-poplar galleries in South Bulgaria [HD 92/43: 92A0 *Salix alba* and *Populus alba* galleries] – category Vulnerable [VU], 07G1 Forests of Oriental plane (*Platanus orientalis* L.) – category Endangered [EN] (Dimitrov and Tzonev 2015, Dimitrov and Tashev 2015a–d, Gogushev 2015, Gussev 2015, Tzonev and Dimitrov 2015). Riparian zones, such as F9.1 Temperate and boreal riparian scrub – category Least Concern [LC], F9.2 *Salix* fen scrub – category Near Threatened [NT], F9.3 Mediterranean riparian scrub – category Least Concern [LC], G1.1 Temperate and boreal softwood riparian woodland – category Near Threatened [NT], G1.2a *Alnus* woodland on riparian and upland soils – category Least Concern [LC], G1.2b Temperate and boreal hardwood riparian woodland – category Endangered [EN], G1.3 Mediterranean and Macaronesian riparian woodland – category Vulnerable [VU], etc. (Janssen et al. 2016), are also considered in the European Red List of

Habitats.

Riparian forest and scrub vegetation are widely distributed across the territory of Bulgaria but still, their syntaxonomic diversity has not been entirely studied. Still, significant knowledge gap exists concerning the riparian vegetation distribution. This paper aims to explore the floristic composition, syntaxonomy, and distribution patterns of high-rank syntaxa of riparian forest and scrub vegetation in Bulgaria. This is the first attempt at a comprehensive analysis of all currently available data on riparian shrub and forest vegetation in Bulgaria, as well as a representation of the syntaxa distribution on a map.

The specific objectives of this study are: (1) to describe the distribution and syntaxonomic diversity of high-rank syntaxa (classes, orders and alliances) of ri-

parian shrubland and forest vegetation in Bulgaria, (2) to characterise the species composition, vegetation structure and ecological preferences of established alliances, and (3) to identify the main threats to the studied vegetation.

Materials and Methods

Study area

The study covers the entire territory of Bulgaria. The subject of the research is vegetation along water bodies – riparian zones. It encompasses both natural and artificial aquatic basins, including rivers, lakes, and reservoirs, as well as water-logged areas with a high groundwater level (Fig. 1).

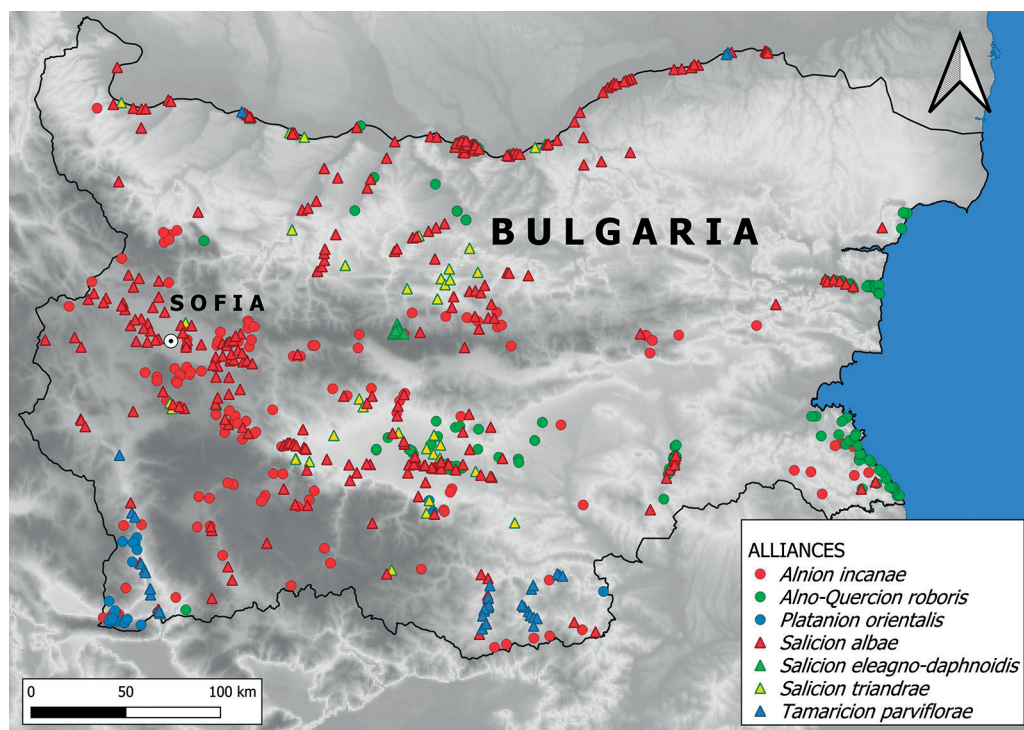


Fig. 1. Map of sampled plots across Bulgaria.

Note: circles – class *Alno glutinosae-Populetea albae*; triangles – class *Salicetea purpureae*.

The altitudinal range of the study sites is from 1 to 1230 m. This study focused on azonal vegetation, which is not principally determined by regional climatic conditions. Periodic or prolonged soil saturation in riparian habitats may lead to anaerobic conditions, which represent an important abiotic factor, influencing the distribution patterns of riparian forests and shrublands. However, such conditions are not uniform and vary depending on hydrological regime, soil type, and topography.

Data sampling and analysis

We gathered 222 published vegetation plots (relevés) from 15 literature sources, presenting riparian shrubland and forest vegetation from Bulgaria (Stoyanoff 1937, 1948; Ganchev 1965; Velchev 1971; Dimitrov and Kosev 1987; Apostolova and Meshinev 2000; Apostolova et al. 2001; Pavlov and Dimitrov 2002; Tzonev 2009a; Pachedzhieva 2011; Dimitrova 2017; Marinov et al. 2017; Glogov et al. 2020; Georgiev 2021). Most of the data were collected following the Braun-Blanquet approach, but older data were according to the Dominant approach and was transformed using the method of Meshinev and Apostolova (2002). Additionally, during the 2011–2024 field seasons, 683 relevés were collected (Fig. 1) following the Braun-Blanquet approach (Westhoff and van der Maarel 1973). The plot size was 60–78 m² for shrubland and 100–600 m² for forest vegetation, as recommended in the relevant literature (Chytrý and Otýpková 2003). All relevés were stored in the Balkan Vegetation Database (Vassilev et al. 2020). For each relevé, full species list of vascular plants and layer structure were recorded. Additionally, information about abiotic characteristics of the environment (elevation, exposure, slope, soil

depth, and bedrock type) was collected.

Altitude and location were recorded using a Garmin eTrex Vista device, while the aspect of the slope was determined with a compass for each relevé. Soil depth was estimated in three categories: (1) shallow (<10 cm), (2) moderately deep (10–20 cm), and (3) deep (>20 cm).

The numerical classification was performed by PC-ORD (McCune and Mefford 1999) and JUICE 7.0 (Tichý 2002) software packages. Sørensen (Bray-Curtis) was used as distance measure and similarity was calculated by Ward's clustering method. A square-root transformation was applied to the species cover values converted to percentages.

Diagnostic species were identified using the Phi-coefficient (Chytrý et al. 2002). Only statistically significant Phi values, evaluated with Fisher's exact test ($p < 0.05$), are presented in the synoptic table (Table A1 in the Appendix). All clusters were standardised to equal sizes. The threshold value for a species to be considered diagnostic was set at a Phi-coefficient ≥ 0.4 (multiplied by 100) in the synoptic table. The species in the synoptic table have been ordered according to decreasing in the fidelity values. The diagnostic role of the species has also been considered according to available literature sources.

For 35 relevés, containing a heterogeneous group of diagnostic species and not fulfilling cluster definition criteria, the Positive Frequency–Fidelity Index (Tichý 2002) was applied for assignment to one of the defined vegetation units.

The 'constancy' of species was represented by percentages in the synoptic table. Species with cover above 50 % at least in 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 used as an indirect ordination technique using the CANOCO 4.5 software package (ter Braak and Smilauer 2002) to reveal the major environmental gradients shaping the floristic composition. Square-root transformation and down-weighting of rare species have been applied. The ecological conditions of habitats among alliances were assessed using the Ecological Indicator Values for Europe (EIVE) 1.0 (Dengler et al. 2023) passively projected onto the ordination space. The significance of correlations between mean EIVs and samples scores along the ordination axes was calculated using the modified permutation test elaborated by Zelený (Zelený and Schaffers 2012) and implemented in the stand-alone application MoPeT (Zelený 2015).

The nomenclature of the vascular plants follows Stoyanov et al. (2022) and has subsequently been standardised according to Euro+Med PlantBase (Euro+Med 2006–2020). Floristic elements were interpreted according to Assyov and Petrova (2012) and species life forms were determined according to Dřevojan et al. (2023). The nomenclature of the high-rank syntaxa was harmonised with Mucina et al. (2016). In addition, bryophytes and lichens were excluded from the species composition because they had not yet been identified for all relevés.

Results and Discussion

Based on the numerical analysis, the syn-taxonomic diversity of shrubland and forest riparian vegetation was represented by 2 classes, 4 orders, and 7 alliances. The syntaxonomic scheme proposed:

1. Class ***Alno glutinosae-Populetea al-***

bae P. Fukarek and Fabijanić 1968

1.1. Order ***Populetea albae*** Br.-Bl. ex Tchou 1949

1.1.1. Alliance ***Platanion orientalis*** I. Kárpáti and V. Kárpáti 1961

1.2. Order ***Alno-Fraxinetalia excelsioris*** Passarge 1968

1.2.1. Alliance ***Alnion incanae*** Pawłowski et al. 1928

1.2.2. Alliance ***Alno-Quercion roboris*** Horvat 1950

2. Class ***Salicetea purpureae*** Moor 1958

2.1. Order ***Salicetalia purpureae*** Moor 1958

2.1.1. Alliance ***Salicion albae*** Soó 1951

2.1.2. Alliance ***Salicion eleagno-daphnoidis*** (Moor 1958) Grass 1993

2.1.3. Alliance ***Salicion triandrae*** T. Müller and Görs 1958

2.2. Order ***Tamaricetalia ramosissimae*** Borza and Boşcaiu ex Dolţu et al. 1980

2.2.1. Alliance ***Tamaricion parviflorae*** I. Kárpáti and V. Kárpáti 1961

All. ***Platanion orientalis*** I. Kárpáti and V. Kárpáti 1961 (Table A1, Cluster 4, 39 relevés)

Constant species: *Urtica dioica*, *Geum urbanum* L., *Rubus caesius*, *Clematis vitalba* L., *Geranium robertianum* L., *Hedera helix* L., *Dactylis glomerata*

Dominant species: *Platanus orientalis* L., *Rubus caesius*

Distribution and ecology: The alliance *Platanion orientalis* includes *Platanus orientalis* gallery forests distributed in the eastern Mediterranean region (Mucina et al. 2016), i.e. within the territories of Italy, Albania, Bulgaria, Greece, North Macedonia, Turkey and Crete (Mandžukovski et al. 2021). In Bulgaria, its phytocoenoses are found in the southern part of the country (southwestern Bulgaria

– along the Struma and Strumeshnitsa rivers and their tributaries, Thracian plain
– near Topolovo village, as well as in the Eastern Rhodopes).

The forests of *Platanion orientalis* form galleries along the riverbed or very rarely at waterlogged sites or gorges with higher soil moisture and consistent water flow. These forests are more often found in the mountainous and semi-mountainous areas and more rarely in the lowlands. The terrain is flat or slightly inclined (the slope was usually between 5–7 °). In some plots near springs in Belasitsa, Vlahina and Ograzhden mountains, the slopes were steeper up to 15–30 °. The expositions were diverse. The phytocoenoses colonise gravelly and sandy terrains along the riverbeds where the soils were moderately deep, alluvial and alluvial-deluvial, and remain well-moistened most of the year. During the spring months (April–June), when rainfall and snowmelt reach their peak, river water levels rise and flooding frequently occurs. In contrast, the summer months are typically characterised by drought and low water levels.

This alliance represents the riparian vegetation with sub-Mediterranean features and is restricted to the southern part of the country. The regional climate, characterised by rising temperatures and decreasing rainfalls, combined with prolonged droughts periods, has led to processes of xerophytisation and resulted in the occurrence of xerophytic and moesoxerophytic species originating from adjacent habitats.

The forests of this alliance are under strong anthropogenic pressure, leading to changes in their structure, functions, and degradation processes. Other factors exerting negative impacts include logging, grazing by domestic animals, pollution, and invasive species, such as *Bidens*

tripartita L., *B. cernua* L., *Impatiens glandulifera* Royle, *Amorpha fruticosa* L., *Robinia pseudoacacia* L. as well as changes in river hydrological regimes and climate change.

The phytocoenoses of this alliance are included in Directive 92/43/EEC as habitat type 92C0 *Platanus orientalis* and *Liquidambar orientalis* Mill. woods (*Platanion orientalis*). They are also included in the Red Data Book of Bulgaria (Gogushev 2015) with code 07G1 Forests of Oriental plane (*Platanus orientalis*). The assessment of the distribution and condition of *Platanus orientalis* habitats was based on the available phytocoenological data used in the present analyses. The distribution range of this vegetation type appears to be broader within the territory of Bulgaria, and further investigations are needed along Mesta, Bachkovska and Sandanska Bistritsa rivers, as well as in the Eastern and Central Rhodopes.

Vegetation description and syntaxonomy: The phytocoenoses were characterised by a closed horizontal structure and moderately to highly species-rich communities (the average species number was 45). The vertical structure includes a tree, shrub and herbaceous layer, as well as a moss layer in certain plots. The total cover was 90–100 %. The tree layer had a cover of 80–100 % (94 % on average). The dominant species was *Platanus orientalis*. Other tree species occurring in the plots included *Carpinus betulus* L., *Fagus sylvatica* L., *Juglans regia*, *Prunus cerasifera*, *Castanea sativa* Mill., and *Alnus glutinosa*. Apart from the shrub species included the tree layer, the shrub layer also included *Crataegus monogyna*, *Corylus avellana* L., *Tilia platyphyllos* Scop., nom. cons., *Sambucus nigra* L., *Cornus mas* L., *Clematis vitalba*, *Rubus caesius* among others. The shrub layer

had a cover of 10–90 % (50 % on average). The herb layer was well developed with a cover of 10–90 % (59 % on average). The species with higher cover and abundance (over 10 %) were *Brachypodium sylvaticum*, *Urtica dioica*, *Parietaria officinalis* L., *Circaea lutetiana* L., *Dryopteris filix-mas* (L.) Schott, *Hedera helix*, and *Aegopodium podagraria*. Mosses occurred in all plots, but their participation varied between 3 and 60 % (mean 9 %).

The analysis of the life-form composition in the phytocoenoses revealed a predominance of hemicryptophytes (44 % of all species), followed by phanerophytes (31 %). On the other hand, based on species cover and abundance data, phanerophytes dominated in the species composition with 83 %, whereas hemicryptophytes and hemicryptophytes-geophytes were represented by 43 % and 15 %, respectively. Euro-Asiatic (23 % of all species), sub-Mediterranean (12 %), European (11 %) and Boreal (10 %) floristic elements dominated in the species composition. The comparison of species cover and abundance revealed the prevalence of Mediterranean elements (82 %), followed by Euro-Asiatic (32 %), European (18 %), Boreal (16 %) and sub-Mediterranean elements (14 %).

The phytocoenoses of the *Platanion orientalis* alliance in Bulgaria have been studied by Pedashenko et al. (2015). Their syntaxonomic diversity is represented by the association *Petasito hybridi-Platanetum orientalis*.

All. *Alnion incanae* Pawłowski et al. 1928 (Table A1, Cluster 3, 142 relevés)

Constant species: *Urtica dioica* L., *Geum urbanum* L., *Brachypodium sylvaticum* (Huds.) P. Beauv., *Galium aparine* L., *Rubus caesius* L., *Geranium robertianum* L., *Dactylis glomerata* L.

Dominant species: *Alnus glutinosa*

(L.) Gaertn., *A. incana* (L.) Moench, *Rubus caesius*, *Urtica dioica*

Distribution and ecology: The phytocoenoses of this alliance were widely spread on the territory of the country, predominantly in the mountainous and semi-mountainous areas. There are limited literature sources for this vegetation type (Stoyanoff 1937, Ganchev 1965, Apostolova and Meshinev 2000, Pachedzhieva 2011). During the last 5–7 years, a total of 138 relevés from this alliance were collected, with the areas more densely studied being the central, southern and western regions of the country (Fig. 1).

The forests of this alliance usually occur in the upper and middle reaches of rivers. Sometimes they also covered waterlogged flat terrains along water bodies and natural depressions of the relief with high groundwater levels. They were characterised by alterations of the water flow during different years and different seasons of the same year. In Bulgaria, the phytocoenoses were distributed between 5 and 1175 m a.s.l. (average 570 m). The soils were medium deep, alluvial and nutrient-rich. The terrain is predominantly flat or gently sloping, with inclines up to 5 °. In a few plots in mountainous regions, slope reaches 25–50 °.

A major negative influence on the forests of this alliance is related to the fluctuations of the water flow and climate change. During recent years, there has been a decline in the waterflow rates in a large part of the rivers, especially during the summer and autumn months. Such changes lead to successional changes in the plant communities with pronounced processes of xerophytisation, which is expressed through the increase of xerophyte and moesoxerophyte species from the neighbouring phytocoenoses. Other negative factors are pollution with house-

hold and inert waste, alteration of riverbeds, extraction of inert materials (gravel, sand), and invasions from non-native invasive plants.

This vegetation type is protected under Directive 92/43/EEC and represents habitat 91E0 Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* L. (*Alno-Padion* Knapp 1942, *Alnion incanae*, *Salicion albae*). It is also included in the Red Data Book of Bulgaria (Tzonev and Dimitrov 2015) with codes 01G1 Riverside poplar-willow forests, 02G1 Flooded forests of common alder (*Alnus glutinosa*), 03G1 mountain grey alder (*Alnus incana*) galleries and 04G1 Riverside woodlands of Alder (*Alnus* spp.) and Common ash (*Fraxinus excelsior*).

Vegetation description and syntaxonomy: The phytocoenoses of the alliance are characterised by a closed horizontal structure with a total cover of 95–100 %. Their species composition was rich, with a total species number recorded for all plots of 142 and average of 32 species per plot. Three main vegetation layers were formed: tree, shrub, and herbaceous. In certain plots, high tree and low tree, as well as moss layers, were formed. The tree layer has a cover of 70–100 % (85 % on average). The dominant species in most of the plots were *Alnus glutinosa* and *A. incana*. Other tree species found in the tree layer were *Fraxinus excelsior*, *Prunus cerasifera* Ehrh., *Juglans regia* L., *Salix purpurea* L., *S. alba* and *S. euxina*. The shrub layer was formed by the species of the tree layer, but also included species like *Rubus caesius*, *Crataegus monogyna* Jacq., *Prunus spinosa* L., *Ulmus minor* Mill., *Acer campestre* L., *Cornus sanguinea* L. Its cover was 50–100 % (40 % on average). The herbaceous layer was characterised by a rich species composition and cover of 2–100 % (61 % on

average). Mosses participated with a cover of up to 4 % in the plots, where data for them was collected.

The analysis of the life-form composition in the phytocoenoses revealed a predominance of hemicryptophytes (43 % of all species), followed by phanerophytes (26 %) and hemicryptophyte–geophyte species (10 %). On the other hand, based on species cover and abundance data phanerophytes dominated in the species composition with 84 %, whereas hemicryptophytes, hemicryptophyte–geophyte species and phanerophyte–chamaephyte were represented by 37 %, 19 % and 16 %, respectively. Euro-Asiatic (30 % of all species), sub-Mediterranean (16 %), Boreal (12 %), South-Boreal (11 %), Euro-Mediterranean (9 %) and European (8 %) floristic elements dominated in the species composition. The comparison of species cover and abundance revealed the prevalence of Mediterranean-Central Asiatic elements (65 %), followed by Euro-Asiatic (43 %), Boreal (18 %) and Euro-Mediterranean (10 %) elements.

The distribution, ecology and syntaxonomy of the alliance have been studied by Petrova and Tzonev (2020), Alexandrova et al. (2020), Nikolov and Dimitrov (2023). The syntaxonomic diversity is represented by a single association (*Stellario nemorum-Alnetum glutinosae*) and plant communities of *Alnus glutinosa* and *Alnus glutinosa*-*Aegopodium podagraria*.

All. ***Alno-Quercion roboris*** Horvat 1950 (Table A1, Cluster 1, 295 relevés)

Constant species: *Geum urbanum*, *Crataegus monogyna*, *Rubus caesius*, *Brachypodium sylvaticum*, *Galium aparine*, *Dactylis glomerata*, *Acer campestre*, *Urtica dioica*

Dominant species: *Fraxinus angustifolia* Vahl, *Quercus robur*

Distribution and ecology: The flood-

plains within the alliance occur in the lower and middle river flows. A total of 295 relevés have been collected across the country (Fig. 1). This alliance occupies larger areas along the Tundzha, Veleka and Ropotamo rivers, as well as in protected areas (e.g. Balabana, Gorna Topchia and Dolna Topchia reserves). It is distributed as well in central parts of the Danubian plain near the Osam river in the region of Levski town and the villages Gradishte, Tranchovitsa and Batzova mahala (Roussakova and Tzonev 2003). Data for their distribution in other regions of the country, alongside the Batova river and other localities in the Thracian lowland and the Tundzhan Hilly Plain, also exists. Nevertheless, the phytocoenoses in these regions have still not been systematically studied.

They are formed along the floodplains of rivers and during spring and the beginning of summer, they are periodically flooded. Flooding is a key factor for the formation and maintenance of these ecosystems. The climate has a clearly expressed Mediterranean influence at most of the studied localities.

These phytocoenoses have been under prolonged anthropogenic influence, which has led to their destruction over the years and their disjunctive distribution nowadays. Large areas have been turned into agricultural lands. Intensive logging has also restricted the spread of this vegetation type. Other negative factors are changes in the water regime and the hydrological conditions, restriction of flooding, dikes construction and other facilities, invasions by non-native species, as well as xerophytisation.

The phytocoenoses are under the protection of the Directive 92/43/EEC (91F0 – Riparian mixed forests of *Quercus robur*, *Ulmus laevis* Pall. and *Ulmus minor*,

Fraxinus excelsior or *Fraxinus angustifolia* Vahl, along major rivers (*Ulmenion minoris* Oberdorfer 1953)). They are also included in the Red Data Book of Bulgaria (Dimitrov and Tzonev 2015) under code 05G1 Riparian and lowland mixed woodlands and longozes.

Vegetation description and syn-

taxonomy: This vegetation type has a closed horizontal structure and a total cover of 75–100 % (81 % on average) and is moderately species-rich (the average number of species is 22). In some plots, where logging and other successional processes of dying out of the tree species occur, the shrub and herb species have higher cover. Forests are dominated by *Fraxinus angustifolia* and *Quercus robur*. Three well-defined layers form the horizontal structure: tree, shrub, and herb layers. The average cover of the tree layer is 76 %. Other tree species that occur are *Acer campestre*, *Populus alba* L., and *Ulmus laevis*. The shrub layer is well developed with a cover of 5–90 % (36 % on average). Widespread species in this layer are *Ulmus minor*, *Crataegus monogyna*, *Rubus caesius*, *Ligustrum vulgare* L., *Cornus mas*, *C. sanguinea*, *Smilax excelsa* L., and *Euonymus europaeus* L. The herb layer has a cover of 5–100 % (60 % on average). Species with higher cover and abundance are *Viola odorata* L. gr., *Brachypodium sylvaticum*, *Dactylis glomerata*, *Galium aparine*, and *Geum urbanum*. The mosses have a low cover (1–2 %) or are missing.

The analysis of the life-form composition in the phytocoenoses shows predominance of phanerophytes (39 % of all species), followed by hemicryptophytes (34 %) and therophytes (8 %). Based on species cover and abundance data, phanerophytes dominated in the species composition with 70 %, whereas hemic-

ryptophytes and therophytes are represented by 26 % and 13 %. Euro-Asiatic (27 % of all species), sub-Mediterranean (14 %), Euro-Mediterranean (14 %), Boreal (6 %) and South-Boreal (7 %) floristic elements dominated in the species composition. The comparison of species cover and abundance shows the prevalence of sub-Mediterranean (38 %), Euro-Asiatic (35 %), followed by Euro-Mediterranean (18 %), and European (6 %) elements.

So far, the syntaxonomy and ecology of this alliance have been investigated by Pavlov and Dimitrov (2002), Roussakova and Tzonev (2003), Dimitrov et al. (2005), and Georgiev (2021). Its syntaxonomic diversity is represented by the association *Smilaco excelsae-Fraxinetum oxycarpae* (Soó 1957) Pavlov et Dimitrov 2002.

All. ***Salicion albae*** Soó 1951 (Table A1, Cluster 2, 314 relevés)

Constant species: *Urtica dioica*, *Rubus caesius*

Dominant species: *Salix alba* L., *S. euxina*, *S. purpurea* L., *Rubus caesius*

Distribution and ecology: These phytocoenoses are more common in the plains and lowlands of the country and occur more rarely in the semi-mountainous regions. They are found at elevations between 11 to 1200 m a.s.l. (330 m on average). Forests are formed mainly as a thin strip along the riverbanks and sometimes also in places where there is a high level of underground water or water retention occurs. They are widespread along the big rivers (such as Danube, Maritsa, Tundzha, Osam, Yantra, Struma, Mesta). The ecosystems are adapted to periodic flooding. Most frequently, it is the lowest river terrace within 2–3 m of the water flow that gets flooded. The terrains are more often even with a slight incline of up to 2–3°. The soils are alluvial, shallow to moderately deep, clay and nutrient-rich.

During the last few years, flooding periods occur more rarely and their length is decreasing. This tendency has a considerable negative influence on the forests, leading to tree drying and xerophytisation of the species composition.

Apart from climate change, other factors, which have a negative influence on this vegetation type, are logging, alterations in the hydrological regime, sand and aggregates extraction, invasions by alien species (*Amorpha fruticosa* L., *Acer negundo* L., *Sicyos angulatus* L., *Phytolacca americana* L., *Bidens* (*B. frondosa* L., *B. tripartita*, *B. cernua*), *Erigeron annuus*, *Conyza canadensis*, etc.). In some phytocoenoses, these species are co-dominants and in rarer cases, they dominate the communities. For example, in the *Amorpho-Salicetum albae* association, *Amorpha fruticosa* has a cover of 50–100 %.

The phytocoenoses are protected under 92/43/EEC Directive (*91E0 Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*), 92A0 *Salix alba* and *Populus alba* galleries). They are also included in the Red Data Book of Bulgaria (Tzonev and Dimitrov 2015) under the code 01G1 Riverside poplar-willow forests.

Vegetation description and syntaxonomy: The phytocoenoses of this alliance have a closed horizontal structure and are moderately species-rich (average number of species per plot is 22). The total cover is 30–100 % (96 % on average). Three layers are formed in the vertical structure: tree, shrub, and herbaceous. The most frequent dominant species in the tree layer are usually willow species – *Salix alba*, *S. euxina*, *S. purpurea*, *Salix × rubens* and more rarely *Populus alba*, *P. nigra*, *Acer negundo*. In most of the sample plots, trees form a layer with a cover

of 70–100 % (79 % on average). Nevertheless, in some plots, trees are degraded due to various disturbances, or are in the beginning stages of their formation and only individual trees are found. The shrub cover varies widely between 5–100 % (54 % on average). Other shrub species found in this layer were *Amorpha fruticosa*, *Rubus caesius*, *Crataegus monogyna*, *Ligustrum vulgare*, *Cornus sanguinea*, etc. The herb layer is diverse, and the species richness and cover are determined by the level of shading. It has an average cover of 56 %. The most widely spread species are *Urtica dioica*, *Carex remota* L., *Lythrum salicaria* L., *Ranunculus repens* L., *Dactylis glomerata*, among others.

The analysis of the life-form composition of the phytocoenoses shows predominance of hemicryptophytes (34 % of all species), followed by phanerophytes (25 %), therophytes (17 %) and hemicryptophytes-geophytes (9 %). Based on cover and abundance species data, phanerophytes dominated in the species composition with 77 %, whereas hemicryptophytes and therophytes were represented by 27 % and 12 %, respectively. Euro-Asiatic (31 % of all species), Boreal (18 %), Euro-Mediterranean (9 %) and South-Boreal (7 %) floristic elements dominated in the species composition. The comparison of species cover and abundance shows the prevalence of Euro-Asiatic elements (70 %), followed by Boreal (18 %), Adventive (9 %) and Euro-Siberian (8 %) elements.

On the territory of the country, the alliance has been studied by Tzonev (2009a,b), Tzonev et al. (2009), and Grigorov et al. (2023a, 2023b). The syntaxonomic diversity is represented by the *Amorpho-Salicetum albae* association.

All. **Salicion eleagno-daphnoidis** (Moor 1958) Grass 1993 (Table A1, Cluster 7, 17

relevés)

Constant species: *Dactylis glomerata*, *Crataegus monogyna*, *Salix alba*

Dominant species: *Salix eleagnos*

Distribution and ecology: The phytocoenoses of this alliance are represented by only 17 relevés from the region of the north-eastern foothills of Central Stara Planina Mountain, along banks of the rivers Cherni Osam, Vidima, Beli and Cherni Vit in their middle and upper reaches. Their stands consist of large alluvial cones of rock and gravel material formed through frequent sedimentation. The phytocoenoses form small patches as islands along rivers with a size of 2–50 m². They occur at slightly inclined terrains with slopes of up to 8–10 ° and diverse exposures. The soils are moderately deep to shallow and are rich in gravel.

According to Directive 92/43/EEC this vegetation is included in the habitat 3240 Alpine rivers and their ligneous vegetation with *Salix elaeagnos*.

Vegetation description and syntaxonomy:

Phytocoenoses are characterised by semi-open to closed horizontal structure. They form three layers in the vertical structure - tree, shrub and herbaceous. The total cover of the tree layer varies between 30 and 70 % (52 % on average). The most abundant species were *Salix eleagnos*, *S. fragilis*, *Fraxinus excelsior*, *Acer pseudoplatanus* L. The shrub layer is formed by the undergrowth of the aforementioned species, but also by *Rubus hirtus* aggr., *Clematis vitalba*, *Crataegus monogyna*, *Acer campestre*, *Cornus sanguinea*, and *Corylus avellana*. Its total cover was 15–50 % (26 % on average). The formation of the herb layer is in direct correlation to the degree of shading and its cover varies between 30 and 40 % (35 % on average). Frequently found species were *Chrysosplenium alternifolium* L.,

Scirpus sylvaticus L., *Angelica sylvestris* L., *Ajuga reptans* L., *Helleborus odoratus* Willd., *Hedera helix*, among others.

In the life-form spectrum of the phytocoenoses, phanerophytes (40 % of all species) predominated, followed by hemicryptophytes (33 %), hemicryptophytes-geophytes (8 %) and geophytes (6 %). On the other hand, based on species cover and abundance data, phanerophytes dominated the species composition by 73 %, whereas hemicryptophytes and hemicryptophytes-geophytes were represented by 28 % and 11 %, respectively. Euro-Asiatic (18 % of all species), South-Boreal (16 %), sub-Mediterranean (15 %) and Euro-Mediterranean (12 %) floristic elements dominated the species composition. The comparison of species cover and abundance shows the prevalence of sub-Mediterranean elements (45 %), followed by Euro-Asiatic elements (41 %) and South-Boreal (15 %) elements.

The phytocoenoses were not very influenced by anthropogenic activities. The only research on the syntaxonomic diversity of the alliance in the country has been carried out by Kalníková et al. (2014, 2021) and Nikolov and Dimitrov (2023). Up to now the syntaxonomic diversity of this alliance is represented by 2 associations (*Saponario officinalis-Salicetum purpureae* Tchou 1948, *Salicetum eleagno-purpureae* Sillinger 1933) and 3 plant communities: *Salix purpurea* and *Calamagrostis pseudophragmites* (Haller f.) Koeler; *Salix elaeagnos* and *Dipsacus laciniatus* L.; *Salix elaeagnos*, and *Petasites hybridus*.

All. ***Salicion triandrae*** T.Müller et Görs 1958 (Table A1, Cluster 6, 58 relevés)

Constant species: *Galium aparine*

Dominant species: *Amorpha fruticosa*, *Anisantha sterilis* (L.) Nevski, *Salix triandra* L., *Elytrigia repens* (L.) Nevski

Distribution and ecology: The phytocoenoses of this alliance occur along riverbanks and waterlogged habitats forming a narrow strip approximately 5–6 m near the forests of *Salix alba*, *S. euxina*. In the country, the alliance has been recorded along the Danube and Maritsa rivers and their tributaries, standing water bodies and places with high underground waters. In some places, they covered smaller plots of 30–60 m², while along the riverbanks they formed bigger stripes. They get flooded during the spring freshet, which results in the deposition of sediments and nutrients and leads to the formation of unstable substrates. During the summer and autumn months, there are low tides, which contribute to the extreme environment. Communities are often found on river islands formed during periods of low water.

Unlike the phytocoenoses of the *Salicion eleagno-daphnoidis* alliance, the communities of *Salicion triandrae* develop on flat terrains with sandy or clay soils along the middle and lower course of rivers.

The factors having a negative influence on this vegetation type are linked to the anthropogenic activities (changes in the hydrological regime of water bodies and the regulation of the water flow, littering and logging of the neighbouring forest phytocoenoses, which leads to mechanical disturbances of the shrub vegetation), as well as to the invasion of alien species and climate change.

Vegetation description and syntaxonomy: This vegetation type comprises mostly shrublands and, more rarely, transitional phytocoenoses between shrublands and forests with semi-open to closed horizontal structure. The total cover was 60–100 % (83 % on average). A shrub layer is formed with a height of 2–4 m and the dominant species were *Salix triandra*

and *Amorpha fruticosa*. Other shrubs also occurred in the phytocoenoses, such as *Salix alba*, *S. euxina*, *Populus nigra* L., *Rubus caesius*, *Cornus sanguinea*, *Clematis vitalba*, and *Humulus lupulus* L. The herbaceous layer was missing or had a low cover of up to 8–10 % in some of the phytocoenoses, which have been recently flooded. In those that have not been flooded in recent years, herb layer was formed with a cover of 30–100 % (62 % on average). Species with a higher cover and abundance are *Anisantha sterilis*, *Elytrigia repens*, *Galium aparine*, *Urtica dioica*. Some alien invasive species, such as *Amorpha fruticosa*, *Bidens tripartita*, *B. frondosa*, *Erigeron annuus* (L.) Desf., *E. canadensis* L. also occurred in some stands. Only *A. fruticosa* forms phytocoenoses where it is a dominant species, while the other species have a cover of up to 1–2 %.

The analysis of the life-form composition of the phytocoenoses revealed the predominance of hemicryptophytes (28 % of all species), followed by therophytes (26 %), phanerophytes (15 %) and hemicryptophytes-geophytes (12 %). On the other hand, based on species cover and abundance data, therophytes dominated the species composition with 31 %, whereas phanerophytes, hemicryptophytes and hemicryptophyte-geophytes were represented by 20 %, 12 %, and 11 % respectively. Euro-Asiatic (26 % of all species), Boreal (12 %), Euro-Mediterranean (11 %), Euro-Siberian (8 %), and Adventive (8 %) floristic elements dominated the species composition. The comparison of species cover and abundance shows the prevalence of Adventive elements (68 %), followed by Euro-Siberian (23 %), Euro-Asiatic (21 %), South-Boreal (9 %), and Boreal (7 %) elements.

The syntaxonomic diversity of the al-

liance has not been studied within the country.

All. ***Tamaricion parviflorae*** I. Kárpáti and V. Kárpáti 1961 (Table A1, Cluster 5, 40 relevés)

Constant species: –

Dominant species: *Tamarix tetrandra* M. Bieb., *T. smyrnensis* Bunge, *Anisantha sterilis*, *Urtica dioica*, *Rubus caesius*

Distribution and ecology: The phytocoenoses of this alliance occur mainly in the southern part of the country along the lower courses of Struma, Maritsa, Arda, Krumovitsa, Varbitsa rivers and their tributaries. They also covered more limited areas along the Danube River. This vegetation type was represented by shrublands or shrubland-forests phytocoenoses and is formed in places of degraded communities of *Salix alba*, *S. fragilis*, *S. purpurea*, *Populus alba*, and *P. nigra*, or between existing ones along riverbanks. In some places, the communities of this alliance were also found in the depressions along the larger rivers, which periodically flooded or had high levels of underground water.

The terrains are flat, and the phytocoenoses predominantly occur in sections of the rivers where they overflow. During the spring months, periodic flooding is characteristic and usually followed by a prolonged drought during summer months. Soils were shallow to moderately deep, sandy and stony, or less frequently clay. Some of the plots are found on slightly saline soils.

According to Gushev (2015), limited areas of this alliance also occur along the Black Sea coast (Balchik region), as well as the Danube River and the Ropotamo deltas. These localities are still not studied, and additional research on the distribution of the phytocoenoses of the alliance in the country is necessary.

According to Directive 92/43/EEC, this vegetation is included in habitat 92D0 Southern riparian galleries and thickets (*Nerio-Tamaricetea* and *Securegion tinctoriae*) and in the Red Data Book of Bulgaria (GusseV 2015) as habitat 31F9 Riverside *Tamarix* (*Tamarix* spp.) stands.

The main factors exerting a negative influence on the alliance are climate change, decrease rainfall, changes in the hydrological regime, grazing, logging of forest and shrub vegetation and clearing of riverbanks, as well as wildfires, littering, and alien species invasions.

Vegetation description and syntaxonomy: The phytocoenoses were characterised by a closed horizontal structure and a total cover of 60–100 % (95 % on average). A shrub layer was formed by including individual trees, such as *Salix alba*, *S. fragilis*, *S. purpurea*, and *Populus alba* among the shrubs. The dominant species in the shrub layer were *Tamarix tetrandra*, *T. smyrnensis*, and *Salix amplexicaulis* Bory. Due to xerophytisation processes in some plots, the cover of *Rubus caesius* increased. The higher level of illumination compared with other riparian phytocoenoses is the reason for the higher species diversity (the average number of species per plots was 29). The herb layer had a cover of 10–100 % (56 % on average). Widespread species included *Anisantha sterilis*, *A. tectorum* (L.) Nevski, *Cynodon dactylon* (L.) Pers., *Chondrilla juncea* L., and *Elytrigia repens*, among others.

The analysis of the life-form composition of the phytocoenoses revealed the predominance of hemicryptophytes (36 % of all species), followed by therophytes (33 %), phanerophytes (12 %) and hemicryptophytes-geophytes (8 %). On the other hand, based on species cover and abundance data, phanerophytes domi-

nated the species composition with 72 %, whereas therophytes and hemicryptophytes were represented by 25 % and 22 %, respectively. Euro-Asiatic (30 % of all species), Euro-Mediterranean (10 %), Euro-Siberian (10 %) and Boreal (8 %) floristic elements dominated the species composition. The comparison of species cover and abundance shows the prevalence of Euro-Asiatic elements (58 %), followed by Mediterranean (38 %), Euro-Siberian (17 %) and Boreal (11 %) elements.

On the territory of the country, the syn-taxonomic diversity of the alliance has not been studied so far.

Ecological characteristics, structure of phytocoenoses, and vegetation-environment relationships

Considering the DCA diagram (Fig. 2), the first axis may be related to the complex influence of continentality and light, and the second axis to moisture amount. The alliances *Tamaricion parviflorae*, *Salicion triandrae*, and *Alno-Quercion roboris* occurred at lower elevations in the lower and middle courses of the rivers. In some of them (all. *Tamaricion parviflorae* and *Alno-Quercion roboris*) there was also a stronger Mediterranean influence. In addition, in phytocoenoses of *Tamaricion parviflorae* and *Salicion triandrae* alliances, the shrubland structure and the less expressed shading effect predetermined higher levels of illumination and xerophytisation.

On the other hand, the phytocoenoses of the alliances *Alnion incanae* and *Salicion eleagno-daphnodis* occurred at higher elevations and under more mesophytic conditions. Such conditions also characterise the alliances *Platanion orientalis* and *Salicion albae*, where the well-devel-

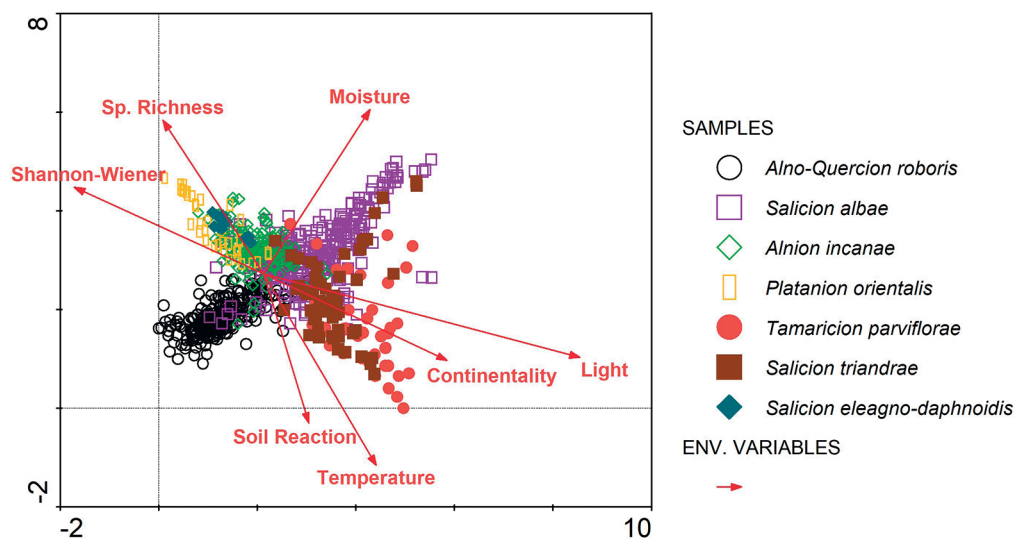


Fig. 2. Detrended correspondence analysis (DCA) was applied to visualise the distribution of the studied alliances within ordination space.

oped tree layer contributes to a stronger shading effect and sustaining of more moesophytic conditions.

When we compared the structure of the phytocoenoses (Fig. 3), we found that the established alliances differed from each other.

The total cover is similar in all phytocoenoses, predominantly between 90–100 %, and is characterised by a closed horizontal structure. When the different layers in the vertical structure (tree, shrub, and herbaceous layer) are compared, significant differences occur between the different vegetation types. The highest cover of the tree layer was found in the alliances *Alnion incanae*, *Platanion orientalis*, and *Salicion albae* (around 75 %), followed by all. *Salicion eleagno-daphnoidis*. The tree layer was missing in the plots of the alliances *Salicion triandrae* and *Tamaricion parviflorae*, as their phytocoenoses were mostly shrublands. Due to this, the cover of the shrub layer was the highest in

both alliances. The other alliances have a shrub layer in the range of 25–50 %. The herb layer has the highest cover in the *Alnion incanae* alliance (with a cover of 75 %) and is the least represented in the *Salicion eleagno-daphnoidis* and *Alno-Quercion roboris* alliances. In the rest of the alliances, the herb layer had a cover of 40–50 %.

Challenges and future perspectives in the research on riparian forests and shrublands in Bulgaria

This is the first comprehensive investigation of this vegetation type in the country, which uncovers the significant ecological diversity of the recorded alliances. Despite the active field studies of this vegetation type and the collection of a huge amount of relevés during the last 4–5 years, there are still significant areas and river systems that are poorly studied. The available data is predominantly restricted

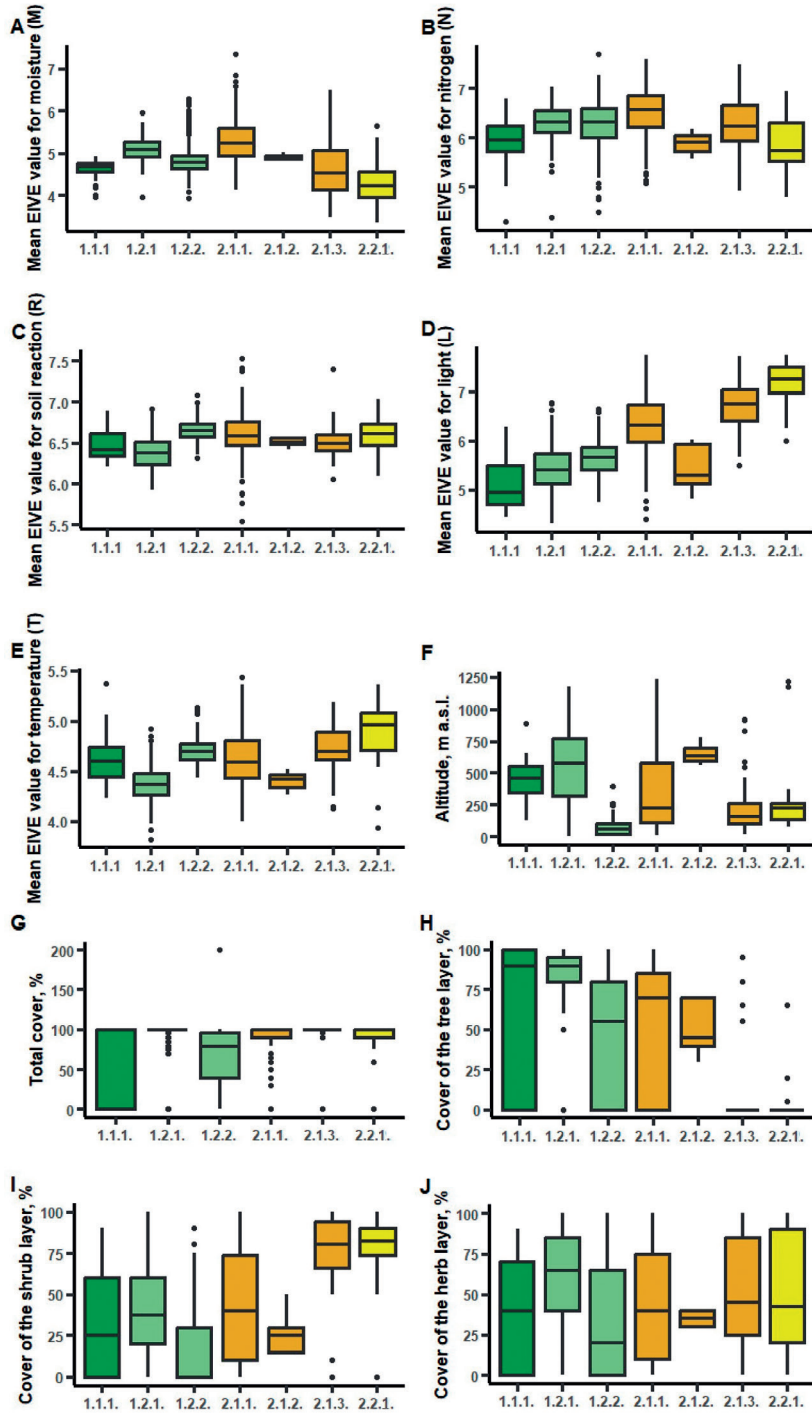


Fig. 3. Distribution of ecological variables across alliances.

Note: Box-and-whisker plots show the distribution of EIVE values, cover values, and altitude for the studied alliances: 1.1.1. All. *Platanion orientalis*, 1.2.1. All. *Alnion incanae*, 1.2.2. All. *Alno-Quercion roboris*, 2.1.1. All. *Salicion eleagno-daphnoidis*, 2.1.3. All. *Salicion triandrae*, 2.2.1. All. *Tamaricion parviflorae*.

to the central, western, and southern parts of the country. In addition, the relevés are irregularly spread between the different alliances. It is necessary to collect data for all alliances, but especially for *Platanion orientalis*, *Alno-Quercion roboris*, *Salicion eleagno-daphnoidis*, *Salicion triandrae*, and *Tamaricion parviflorae*, for which current data are most limited.

This research is the first important step to assessing the distribution, syntaxonomic diversity, ecological characteristics, condition and threats for the different phytocoenoses and habitats of riparian forests and shrublands in the country, based on recent phytocoenological data. The next step in these studies should be connected to establishing the associations and plant communities that represent this vegetation in the country. It would be preferable to collect relevés from at least 80 % of the potential 10 × 10 km grids, where each alliance could be found, before more comprehensive analyses are done. To achieve this for 2025, the collection of field data for the south-eastern part of the country, as well as for the Central and Western Rhodope mountains, Rila, Pirin and along the Danube River, continues. While the larger alliances like *Alno-Quercion roboris*, *Alnion incanae* and *Salicion albae* show significant homogeneity in the data collected so far, for the other alliances, the data is insufficient and/or display greater floristic differences between the relevés.

Four alliances are recorded for the country, but their distribution is still questionable – *Artemisio scopariae-Tamaricion ramosissimae* Simon and Dihoru 1963, *Populion albae* Br.-Bl. ex Tchou 1949, *Salicion cinereae* T. Müller and Görs ex Passarge, and *Fraxino-Quercion roboris* Passarge 1968 (Apostolova 2023). They were not confirmed during this research. It is necessary to purposefully search for

localities where they could be found. Floristic and forestry data could be utilised for this purpose. According to Biurrun et al. (2021) and Preislerová et al. (2022), the distribution of the *Populion albae* alliance is restricted to the Western Mediterranean. Additionally, according to Preislerová et al. (2022), the *Fraxino-Quercion roboris* is also not distributed in the country.

It is necessary to carry out comparative studies with similar vegetation types on the Balkan Peninsula and in South-Eastern Europe. The results of the study by Douda et al. (2016) contribute to the knowledge of this vegetation type at the European level, but they include limited data from the territory of Bulgaria. The investigations of riparian forests and shrublands are important not only from a scientific, but also from an applied perspective and for nature conservation purposes.

Conclusion

The numerical classification of riparian vegetation resulted in the identification of two classes (*Salicetea purpureae*, *Alno glutinosae-Populetea albae*) and seven alliances (*Platanion orientalis*, *Alnion incanae*, *Alno-Quercion roboris*, *Salicion albae*, *Salicion triandrae*, *Salicion eleagno-daphnoidis*, and *Tamaricion parviflorae*). Three alliances (*Alno-Quercion roboris*, *Platanion orientalis*, and *Tamaricion parviflorae*) are composed of forests characterised by Mediterranean influence. Species composition of the studied vegetation was shaped by the continental characteristics of the climate, which influence regimes of ecological factors, such as light and temperature. Water availability and elevation also played important roles in determining vegetation structure and species composition of the riparian forests

and shrublands. Among the major threats and pressures upon this vegetation are water level fluctuations, climate change, especially decreased rainfalls and xerophytisation, alien plant species invasion, and human activities involving riverbank alteration, pollution, logging, grazing.

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Appendix A

Table A1. Synoptic table of shrubland and forest riparian vegetation in Bulgaria.

	Number of clusters													
	1		2		3		4		5		6		7	
	295	Phi	314	C	142	Phi	39	C	40	Phi	58	C	17	Phi
Average species number per relevé														
Fidelity / Constancy														
Diagn. species of all. <i>Alno-Quercion roboris</i>														
<i>Fraxinus angustifolia</i> Vahl	88	81.8	---	8	---	4	---	0	---	0	---	9	---	0
<i>Quercus robur</i> L.	58	68.6	---	3	---	1	---	3	---	0	---	0	---	0
<i>Ulmus minor</i> Mill.	74	63.2	---	12	---	10	---	15	---	0	---	5	---	0
<i>Smilax excelsa</i> L.	29	50.2	---	1	---	1	---	0	---	0	---	0	---	0
<i>Leucojum aestivum</i> L.	21	43	---	1	---	0	---	0	---	0	---	0	---	0
Diagn. species of all. <i>Salicion albae</i>														
<i>Salix alba</i> L.	4	---	40	67	---	23	---	0	17	50	---	14	24.8	59
Diagn. species of all. <i>Alnion incanae</i>														
<i>Alnus glutinosa</i> (L.) Gaertn.	10	---	---	12	67.6	89	---	33	---	3	---	3	---	0
Diagn. species of all. <i>Platanion orientalis</i>														
<i>Platanus orientalis</i> L.	0	---	---	1	---	2	---	100	---	0	---	0	---	0
<i>Castanea sativa</i> Mill.	0	---	---	0	---	1	---	46	---	0	---	0	---	0
<i>Fraxinus ornus</i> L.	0	---	---	2	---	4	---	62.1	---	0	---	0	---	0
<i>Salvia glutinosa</i> L.	0	---	---	1	---	5	---	54.4	---	0	---	0	---	0
<i>Sanicula europaea</i> L.	0	---	---	1	---	3	---	53.9	---	0	---	0	---	0
<i>Pteridium aquilinum</i> (L.) Kuhn	0	---	---	1	7.7	13	---	52.5	---	0	---	0	---	0
<i>Asplenium adiantum-nigrum</i> L.	0	---	---	0	---	0	---	31	---	0	---	0	---	0
<i>Poa nemoralis</i> L.	4	---	---	7	19.1	32	---	52	---	0	---	3	---	0
<i>Circaea lutetiana</i> L.	7	---	---	4	14.8	27	---	51.2	---	5	---	0	---	0
<i>Asplenium trichomanes</i> L.	0	---	---	0	---	1	---	51	---	0	---	0	---	0
<i>Carpinus orientalis</i> Mill.	2	---	---	1	---	2	---	50.8	---	0	---	0	---	0
<i>Cyclamen hederifolium</i> Aiton	0	---	---	0	---	0	---	50.2	---	0	---	0	---	0
<i>Primula acaulis</i> (L.) L.	1	---	---	0	---	2	---	50	---	0	---	0	---	0
<i>Veronica chamaedrys</i> L.	1	---	---	1	---	2	---	49.6	---	0	---	2	---	0

Number of clusters Number of relevés Average species number per relevé	1		2		3		4		5		6		7	
	295		314		142		39		40		58		17	
	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C
Fidelity / Constancy	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C
<i>Rosa arvensis</i> Huds.	---	0	---	0	---	1	49.4	28	---	0	---	0	---	0
<i>Viola alba</i> Besser	---	1	---	1	---	2	49.3	31	---	0	---	0	---	0
<i>Chaerophyllum temulum</i> L.	---	2	---	4	---	8	49	41	---	3	---	0	---	0
<i>Melica uniflora</i> Retz.	---	1	---	1	---	11	48.7	38	---	0	---	0	---	0
<i>Fragaria viridis</i> Weston	---	0	---	0	---	1	46.9	26	---	0	---	0	---	0
<i>Primula veris</i> L.	---	1	---	1	---	1	46.2	26	---	0	---	0	---	0
<i>Parietaria officinalis</i> L.	---	9	---	13	---	18	46	54	---	5	---	2	---	0
<i>Cardamine bulbifera</i> (L.) Crantz	---	3	---	1	---	9	45.5	51	---	0	---	0	19.3	29
<i>Cicerbita muralis</i> (L.) Wallr.	---	1	---	1	---	25	45.3	44	---	0	---	0	---	0
<i>Moehringia trinervia</i> (L.) Clairv.	---	1	---	1	---	2	44.7	26	---	0	---	0	---	0
<i>Saxifraga rotundifolia</i> L.	---	1	---	0	---	3	44.5	28	---	3	---	0	---	0
<i>Veronica urticifolia</i> Jacq.	---	0	---	0	---	1	44.3	23	---	0	---	0	---	0
<i>Aremonia agrimonoides</i> (L.) DC.	---	5	---	1	---	7	44.3	33	---	0	---	0	---	0
<i>Dryopteris filix-mas</i> (L.) Schott	---	0	---	1	---	16	43.8	49	---	0	---	0	13.1	24
<i>Campanula trachelium</i> L.	---	2	---	1	---	2	43.3	26	---	0	---	0	---	0
<i>Galium odoratum</i> (L.) Scop.	---	0	---	1	---	1	42.5	28	---	0	---	0	---	6
<i>Ostrya carpinifolia</i> Scop.	---	0	---	1	---	3	41.6	23	---	0	---	0	---	0
<i>Cardamine impatiens</i> L.	---	0	---	3	---	3	41.2	28	---	0	---	0	---	0
Diagn. species of all. <i>Tamaricion parviflorae</i>														
<i>Tamarix tetrandra</i> M. Bieb.	---	0	---	1	---	1	---	0	74.2	60	---	0	---	0
<i>Salix amplexicaulis</i> Bory	---	0	---	1	---	0	---	0	59.1	40	---	0	---	0
<i>Anisantha tectorum</i> (L.) Nevski	---	0	---	1	---	0	---	0	51.1	30	---	0	---	0
<i>Artemisia campestris</i> L.	---	0	---	1	---	0	---	0	50.5	30	---	0	---	0
<i>Sonchus asper</i> (L.) Hill	---	3	---	3	---	0	---	0	48.1	33	---	0	---	0
<i>Euphorbia platyphyllos</i> L.	---	0	---	1	---	1	---	0	46.9	28	---	0	---	0
<i>Chondrilla juncea</i> L.	---	0	---	1	---	1	---	0	44.9	28	---	2	---	0
<i>Bromus racemosus</i> L.	---	1	---	1	---	1	---	0	44.4	25	---	0	---	0
<i>Anthemis arvensis</i> L.	---	0	---	1	---	0	---	0	43.8	23	---	0	---	0
<i>Medicago minima</i> (L.) L.	---	0	---	0	---	0	---	0	42.5	23	---	2	---	0

	Number of clusters													
	1		2		3		4		5		6		7	
	295		314		142		39		40		58		17	
Average species number per relevé	23		22		32		45		29		15		35	
Fidelity / Constancy	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C
<i>Xanthium strumarium</i> L.	---	0	9.9	14	---	1	---	0	42.2	35	---	3	---	0
<i>Daucus carota</i> L.	---	1	---	1	---	0	---	0	42	25	---	3	---	0
<i>Mellilotus officinalis</i> (L.) Lam.	---	1	---	2	---	1	---	0	40.8	25	---	2	---	0
<i>Cynodon dactylon</i> (L.) Pers.	---	0	---	2	---	1	---	3	40.2	30	4.1	9	---	0
Diagn. species of all. <i>Salicion triandrae</i>														
<i>Amorpha fruticosa</i> L.	---	6	16.7	39	---	4	---	8	---	13	64	88	---	0
<i>Poa angustifolia</i> L.	---	1	---	1	---	0	---	0	---	5	43.8	28	---	0
<i>Anisantha sterilis</i> (L.) Nevski	---	6	---	13	---	13	---	5	18.7	38	42.3	60	---	0
Diagn. species of all. <i>Salicion eleagno-daphnoidis</i>														
<i>Salix eleagnos</i> Scop.	---	0	---	1	---	0	---	0	---	0	---	0	99.4	100
<i>Dipsacus fullonum</i> L.	---	0	---	1	---	0	---	0	---	0	---	0	92.6	88
<i>Acer platanoides</i> L.	---	0	---	1	---	3	---	10	---	0	---	0	92.5	100
<i>Fraxinus excelsior</i> L.	---	1	---	8	---	10	---	3	---	0	---	3	87.8	100
<i>Angelica sylvestris</i> L.	---	4	---	5	---	11	---	15	---	0	---	0	83.5	100
<i>Rubus hirtus</i> aggr.	---	4	---	3	---	11	2.8	23	---	0	---	2	80.8	100
<i>Chrysosplenium alternifolium</i> L.	---	0	---	1	---	4	---	5	---	0	---	0	80.1	76
<i>Carpinus betulus</i> L.	---	4	---	4	1.2	22	---	15	---	0	---	0	80.1	100
<i>Scirpus sylvaticus</i> L.	---	1	---	9	3.7	22	---	0	---	3	---	0	79.9	94
<i>Salix euxina</i> I.V.Belyaeva	---	2	8.8	35	7.9	34	---	3	---	3	---	2	70.1	100
<i>Ajuga reptans</i> L.	---	1	---	1	---	9	---	5	---	0	---	0	68.6	65
<i>Helleborus odoratus</i> Willd.	---	1	---	1	---	2	---	0	---	0	---	0	67.9	53
<i>Juglans regia</i> L.	---	3	---	13	1.7	27	10.3	36	---	0	---	2	65.2	94
<i>Clinopodium vulgare</i> L.	---	1	---	1	---	5	12.3	26	---	0	---	2	63.9	71
<i>Mercurialis perennis</i> L.	---	4	---	1	---	9	---	3	---	0	---	0	63.7	59
<i>Corylus avellana</i> L.	---	22	---	7	1.6	31	12.9	44	---	0	---	0	63.6	100
<i>Geranium macrorrhizum</i> L.	---	0	---	1	---	2	25.1	38	---	0	---	0	61	71
<i>Stellaria media</i> (L.) Cirillo	---	13	---	7	---	4	---	8	14.5	40	---	12	60.2	88
<i>Acer campestre</i> L.	29.6	62	---	7	---	25	---	15	---	0	---	0	58.5	94

	Number of clusters													
	1		2		3		4		5		6		7	
	295	23	314	22	142	32	39	45	40	29	58	15	17	35
Average species number per relevé	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C
Fidelity / Constancy														
<i>Prunus cerasifera</i> Ehrh.	---	16	---	20	9.4	44	---	33	---	3	---	14	58.4	100
<i>Geranium robertianum</i> L.	---	1	---	10	18.4	54	24.8	62	---	5	---	0	58.2	100
<i>Hedera helix</i> L.	11.3	42	---	4	---	16	19.3	51	---	0	---	0	57.6	94
<i>Anemone ranunculoides</i> L.	---	6	---	0	---	0	---	5	---	0	---	0	57.2	47
<i>Lathraea squamaria</i> L.	---	0	---	0	---	0	---	0	---	0	---	0	56.4	35
<i>Ranunculus repens</i> L.	---	8	---	20	12.8	35	---	0	---	5	---	7	54.5	76
<i>Cornus sanguinea</i> L.	4.8	39	---	21	---	33	---	23	---	3	---	19	53	94
<i>Taraxacum officinale</i> aggr.	---	7	---	1	---	4	1.6	13	---	0	---	3	52.9	53
<i>Juncus effusus</i> L.	---	2	---	8	4	15	---	3	---	5	---	0	50.6	53
<i>Agrimonia eupatoria</i> L.	---	3	---	6	---	4	---	5	---	0	---	9	48.5	47
<i>Rumex acetosella</i> L.	---	0	---	0	---	1	---	3	9.5	15	---	0	47.9	41
<i>Veronica anagallis-aquatica</i> L.	---	1	---	5	---	4	---	0	---	8	---	2	47.5	41
<i>Clematis vitalba</i> L.	---	16	---	19	---	35	21.1	64	---	33	---	12	46.3	94
<i>Tussilago farfara</i> L.	---	1	---	1	---	3	---	3	0.4	8	---	2	44.1	35
<i>Morus alba</i> L.	---	3	7.5	15	---	2	---	0	---	0	---	5	43.9	41
<i>Epilobium hirsutum</i> L.	---	1	1.6	11	---	4	---	3	---	8	---	0	43.5	41
<i>Pulmonaria rubra</i> Schott	---	0	---	0	---	2	12.1	15	---	0	---	0	42.9	35
Diagn. species of cl. <i>Aino glutinosae</i>-<i>Populetea albae</i>														
<i>Ulmus laevis</i> Pall.	27.2	22	---	14	---	6	---	0	---	0	---	2	---	0
<i>Melissa officinalis</i> L.	13.6	7	---	0	---	1	---	8	---	0	---	0	---	0
<i>Ficaria verna</i> Huds.	37	27	---	2	---	1	---	10	---	0	---	0	---	0
<i>Populus alba</i> L.	24.9	25	---	15	---	6	---	0	---	8	---	5	---	0
<i>Alliaria petiolata</i> (M. Bieb.) Cavara & Grande	18.8	34	---	9	12.9	28	---	26	---	18	---	2	---	0
<i>Carex pendula</i> Huds.	---	3	---	4	22.6	14	---	5	---	0	---	0	---	0
<i>Equisetum sylvaticum</i> L.	---	0	6.5	14	18.1	22	---	13	---	3	---	7	---	6
<i>Solanum dulcamara</i> L.	---	5	13	16	18.9	20	---	5	---	5	---	2	---	0
<i>Carex remota</i> L.	7.9	22	---	8	36.4	46	---	23	---	3	---	2	---	0
<i>Schedonorus giganteus</i> (L.) Holub	---	2	---	5	32.8	25	---	5	---	3	---	2	---	0

Average species number per relevé	1		2		3		4		5		6		7	
	Number of clusters		Number of relevés		Fidelity / Constancy		Phi		C		Phi		C	
	295	23	314	22	142	32	39	45	40	29	58	15	35	17
<i>Rubus caesius</i> L.	20.2	70	---	57	58	---	---	64	---	35	---	34	---	0
<i>Impatiens glandulifera</i> Royle	---	0	---	1	15.6	4	---	0	---	0	---	0	---	0
<i>Prunus avium</i> (L.) L.	---	1	---	1	24	8	---	0	---	0	---	0	---	0
<i>Rumex sanguineus</i> L.	16.2	31	---	15	14.4	29	---	23	---	8	---	7	---	0
<i>Brachypodium sylvaticum</i> (Huds.) P.Beauv.	29.8	71	---	23	20.7	60	39.7	82	---	10	---	3	---	0
<i>Populus nigra</i> L.	---	1	14.9	14	---	6	---	0	---	10	---	9	---	0
<i>Geum urbanum</i> L.	26.8	76	---	24	25.8	75	21.3	69	---	10	---	9	---	41
<i>Dioscorea communis</i> (L.) Caddick & Wilkin	12.6	22	---	6	---	14	27.4	33	---	5	---	2	---	0
<i>Stellaria nemorum</i> L.	---	3	---	7	22.1	26	11	18	---	13	---	3	---	0
<i>Impatiens noli-tangere</i> L.	---	0	---	3	27.1	24	17.5	18	---	3	---	2	---	0
<i>Scrophularia nodosa</i> L.	---	0	---	2	---	4	34	23	---	3	---	3	---	0
<i>Stachys sylvatica</i> L.	---	2	---	8	29.3	38	32.9	41	---	5	---	0	---	0
Diagn. species of cl. <i>Salicetea purpureae</i>														
<i>Urtica dioica</i> L.	---	60	---	59	26.4	82	---	69	---	33	---	31	---	12
<i>Elymus caninus</i> (L.) L.	---	0	---	3	24.3	11	---	0	---	0	---	2	---	0
<i>Humulus lupulus</i> L.	13.1	29	14	30	---	21	---	3	---	15	---	21	---	0
<i>Salix triandra</i> L.	---	1	6.8	9	7.9	10	---	0	---	5	15	14	---	0
<i>Salix purpurea</i> L.	---	0	24.6	20	---	13	---	0	---	3	---	5	---	0
<i>Lamium maculatum</i> (L.) L.	---	2	---	3	11.2	15	34.2	31	---	5	---	0	---	0
Other frequent species														
<i>Euphorbia amygdaloides</i> L.	---	2	---	1	---	14	40.6	59	---	0	---	2	40.5	59
<i>Crataegus monogyna</i> Jacq.	36.4	74	---	18	---	42	---	13	---	0	---	9	33.5	71
<i>Sambucus nigra</i> L.	8	33	---	12	15.9	41	---	21	---	3	---	2	33.1	59
<i>Lysimachia nummularia</i> L.	5.8	26	---	18	---	26	---	8	---	3	---	14	27.5	47
<i>Dactylis glomerata</i> L.	10.2	56	---	26	6.2	51	---	51	---	40	---	12	21.9	71
<i>Agrostis stolonifera</i> L.	---	3	19.3	32	---	23	---	0	25.7	38	---	10	---	0
<i>Poa trivialis</i> subsp. <i>sylvicola</i> (Guss.) H.Lindb.	11.4	21	---	10	---	18	---	0	19.6	28	---	7	---	0
<i>Lycopus europaeus</i> L.	---	7	19.4	29	19.6	29	---	13	---	5	---	7	---	0
<i>Holcus lanatus</i> L., nom. cons.	---	1	9.8	16	10.6	16	---	5	---	18	---	7	---	0

	1		2		3		4		5		6		7	
	Number of clusters		Number of relevés		Number of relevés		Number of relevés		Number of relevés		Number of relevés		Number of relevés	
	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C	Phi	C
Average species number per relevé	23	23	22	22	32	32	45	45	29	29	15	15	35	35
Fidelity / Constancy														
<i>Artemisia vulgaris</i> L.	---	2	6.9	14	---	8	---	3	23.2	25	---	10	---	0
<i>Galium aparine</i> L.	18.7	71	---	48	---	59	---	46	---	50	---	64	---	0
<i>Rosa canina</i> L.	---	22	---	14	14.7	32	---	31	---	13	---	17	---	0
<i>Aegopodium podagraria</i> L.	---	6	---	16	39.4	62	26.4	49	---	0	---	3	---	18
<i>Elytrigia repens</i> (L.) Nevski	---	3	---	14	---	3	---	0	27.4	40	38.7	50	---	0
<i>Glechoma hederacea</i> L.	---	17	---	16	---	20	---	10	---	0	---	3	---	0

Note: The species are represented by two indicators – the fidelity measure, expressed by the Phi-coefficient (Chytrý et al. 2002), and Constancy, expressed as a percentages. Highly diagnostic species are marked in dark green, whereas diagnostic species are marked in light green.

Influence of European mistletoe (*Viscum album* L.) on crown defoliation in *Populus × canadensis* plantations on coastal dunes along the Central Black Sea coast of Bulgaria

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Thematic field: Forest disturbances and management challenges

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Abstract

The study aimed to examine the relationship between the occurrence of European mistletoe (*Viscum album* L.) and crown defoliation of Canadian poplar (*Populus × canadensis* Moench) plantations established on coastal dunes along the Central Black Sea coast of Bulgaria. A total of 1,158 trees were assessed for infestation level using a four-class visual scale (no, light, moderate, and heavy infestation) and for crown defoliation following the ICP Forest protocol. Nearly half of the trees (49.8 %) were free of mistletoe, while moderate to heavy infestation occurred in less than 10 %. Defoliation was absent or slight in about two-thirds of the trees, whereas severe defoliation was recorded in 8 %. Statistical analyses revealed a positive correlation between mistletoe abundance and crown defoliation, as well as with stem diameter, indicating that larger and taller trees are more susceptible despite the even-aged structure of the plantations. Although mistletoe did not directly cause tree mortality, it significantly contributed to defoliation and may predispose trees to other biotic and abiotic stressors. A decision-tree model identified threshold values of infestation that predict changes in crown condition, offering a practical tool for assessing host-tree health in dune plantations.

Key words: forest health, hemiparasitic plant, host tree health, infestation thresholds, plantation management, poplars, tree size and susceptibility.

Introduction

European mistletoe (*Viscum album* L.) is a major stressor for forest ecosystems. This hemiparasitic plant extracts water and nutrients from its host trees, exhibits high frequency of occurrence, and spreads rapidly across a wide variety of tree species (Barney et al. 2007, Camarero et al. 2019). Understanding the relationship between mistletoe infestation and tree defoliation is crucial not only for ecological research but also for practical forest management (Whitaker et al. 2025). Crown defoliation is an indicator of deteriorating tree health, which may indicate either the direct negative impact of mistletoe – causing water stress and nutrient deficiency – or the indirect effects of other abiotic and biotic factors that weaken the tree's physiological mechanisms (Bussotti et al. 2024, Pawłowicz et al. 2024).

Dobbertin and Rigling (2006) found that trees infected with mistletoe exhibited significantly higher crown transparency than uninfected ones. Lech et al. (2020) reported that in Polish forests, mistletoe infestation affected conifers, mostly such as *Pinus nigra* Arn. and *Abies alba* Mill., though broadleaf deciduous species were also impacted. They found that trees colonised by mistletoe showed significantly greater defoliation than uninfected ones. Kartawik et al. (2023) confirmed that the extensive infestation of pine stands by mistletoe (*Viscum album* ssp. *austriacum* (Wiesb.) Vollm.) has become a serious concern for forest management. Their study in Toruń (Northern Poland) revealed that the likelihood of substantial needle loss rises with an increase in mistletoe numbers, irrespective of tree age. Similarly, research by Whitaker et al. (2025) in Staffordshire, UK, revealed a strong positive correlation between mistletoe density

and crown defoliation in two host species: *Tilia cordata* Mill. and *Acer pseudoplatanus* L.

Recent research emphasises that the size and height of host trees are critical determinants of mistletoe susceptibility and its subsequent physiological impact. Gołębek (2017) found that in urban populations of *Populus nigra* L., *Tilia cordata* and *Robinia pseudoacacia* L., larger and taller trees hosted more mistletoe shrubs, with clear negative correlation between infestation degree and tree vitality. Likewise, González de Andrés et al. (2024) demonstrated that heavily infested trees experience pronounced carbon–water–nutrient imbalances detectable in tree rings, and that dominant individuals tend to become the primary infection sources. Mutlu et al. (2016) also showed that in *Pinus sylvestris* L. stands, the physiological stress caused by *Viscum album* increases under drought, particularly in larger hosts that sustain higher transpirational demand.

Landscape-scale analyses further confirm that tree size is a consistent predictor of infestation risk. Sayad et al. (2017) reported that mistletoe abundance increases with tree diameter and crown height, a pattern linked to bird dispersal behaviour and microclimatic exposure. In addition, Kleszken et al. (2022) found that among several host species, tall poplars exhibited the highest infection frequency, reinforcing the idea that canopy dominance enhances both exposure and parasite load. Similar observations were made by Skrypnik et al. (2020) in Kaliningrad, where urban areas with older, large-crowned trees showed the most intensive mistletoe spread, influenced by both structural and landscape factors. These findings indicate that tree size should be considered a leading predictor of mistletoe impact in plantation and natural stands alike.

In Bulgaria, the relationship between mistletoe presence and the health status of host trees, including crown defoliation, has not been investigated in depth. Studies by Kuleliev and Mitsov (1974), Rosnev and Petkov (2001), and Glogov and Bogdanov (2024) in the Sunny Beach resort area and the town of Nessebar identified poplars (*Populus alba* L., *P. nigra*, *P. deltoides* W. Bartram ex Marshall and *P. × canadensis* Moench) as primary hosts of European mistletoe. These authors observed that mistletoe has a greater negative impact on the health of its hosts as they age, though no specific quantitative data were provided.

The aim of the present study is to examine the relationship between the intensity of European mistletoe (*Viscum album*) infestation and the degree of crown defoliation in *Populus × canadensis* stands established on coastal dune plantations along the Central Bulgarian Black Sea coast. The analysis is based on a single regional dataset and should therefore be interpreted as a case study illustrating local patterns rather than broad generalisations. Particular attention is given to tree size as a potential predictors of infestation severity complementing previous findings from European and Mediterranean contexts.

Material and Methods

Study area

The study was conducted in July–August 2024 on the territory of the State Forestry Enterprise Nessebar. Plantations of Canadian poplar (*Populus × canadensis*, cultivars ‘Regenerata’ and ‘Bachelieri’ and their hybrids) were examined. These plantations dominate in the Natura 2000

protected zone BG0000547 Aheloy–Ravda–Nessebar, specifically within habitat type 2130 Fixed coastal dunes with herbaceous vegetation (‘grey dunes’), covering about 12 km², elevations between 5 and 25 m a.s.l. The central point of the surveyed area is located at 42°39′27.7″ N, 27°42′29.7″ E. Poplar plantations are situated in fragments across the dunes, each ranging in size from 50×50 m to 100×50 m. The predominant tree age in these plantations is between 20 and 40 years. The studied areas fall within forest units 561, 562, and 563 of the current Forest Management Plan (EFA 2019) (Fig. 1). The poplar plantations were artificially established in the second half of the 20th century to stabilise and protect sandy masses and to reduce dune mobility. These stands are not targets of conservation under the Habitats Directive; indeed, the non-native *P. × canadensis* may sometimes inhibit the development of characteristic dune vegetation.

Sampling and measurements

The methodology follows Whitaker et al. (2025). Tree infestation levels were categorised as follows: 0 – No visible infestation, 1 – light (fewer than 10 clusters), 2 – moderate (10–30 clusters), 3 – heavy (more than 30 clusters).

Measurements were taken for each *P. × canadensis* tree with a stem diameter >10 cm: stem diameter at 1.30 m height, number of mistletoe plants, and percentage of crown defoliation according to ICP Forest protocol (Eichhorn et al. 2016). Defoliation was rated on a five-point scale: 0 – no damage (up to 10 % leaf loss); 1 – mild (10–25 %); 2 – moderate (>25–<60 %); 3 – severe (>60–<100 %); and 4 – dead (100 % defoliation). For the purposes of statistical modelling, these

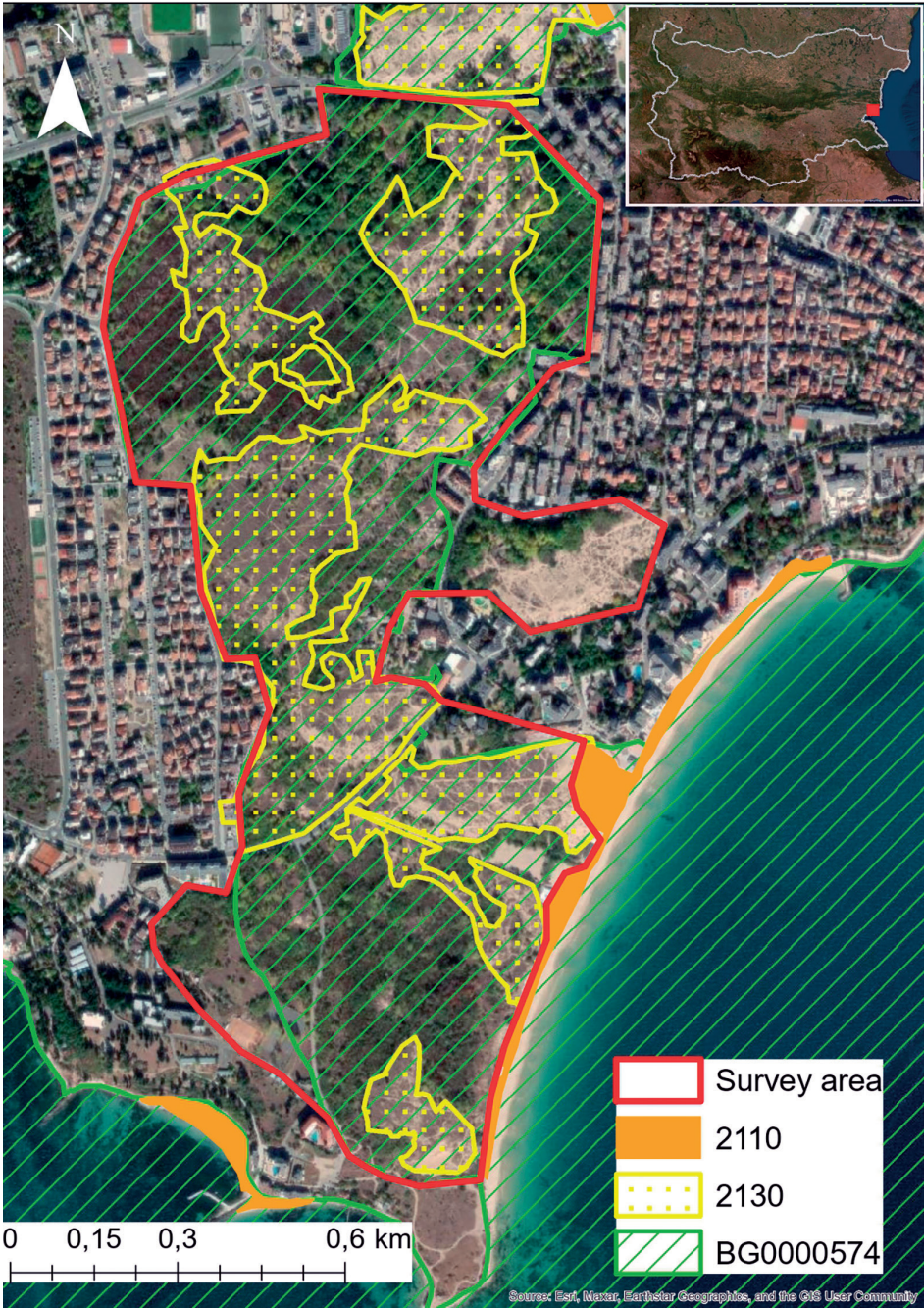


Fig. 1. Study area.

Note: 2130 – habitat type Fixed coastal dunes with herbaceous vegetation ('grey dunes');
2110 – habitat type Embryonic shifting dunes.

classes were coded as NoD (0), LowD (1), MedD (2), StrD (3) and Dead (4). In the case of coppice shoots with 2–3 stems per tree, average values were calculated.

Data analysis

Correlation and regression analyses were done using R (R Core Team 2024), with data preparation following Wickham (2007). One question arising from the study's aim is whether threshold values exist for the number of mistletoe individuals (clusters) that determine the extent of defoliation. To address this question, a decision tree procedure was applied. This method requires the specification of a classifying variable (in this case, the number of mistletoe individuals) and the examination of groupings across its different values.

Based on the number of mistletoe individuals, two comparative groups were distinguished for subsequent analyses: Group A: trees with no or a single mistletoe cluster ($N \leq 1$); Group B: trees with more than one mistletoe cluster ($N > 1$). These groups were used in regression and ANOVA tests to determine whether defoliation–diameter relationships differed depending on infestation level.

Statistical significance was evaluated at conventional probability thresholds ($p < 0.05$, $p < 0.01$, and $p < 0.001$). In the figures and tables, these levels are indicated using asterisks in accordance with R software conventions:

- $p < 0.05$ – * (single asterisk) – statistically significant;
- $p < 0.01$ – ** (double asterisks) – highly significant;
- $p < 0.001$ – *** (triple asterisks) – very highly significant.

Non-significant results ($p \geq 0.05$) are indicated as 'ns' or left unmarked. This

notation follows the standard output format of the R statistical environment and facilitates a consistent interpretation of all regression, ANOVA, and post-hoc tests presented in the manuscript.

The decision tree models decision-making processes through a hierarchical structure resembling a tree. Each node represents a condition or splitting criterion, while the branches indicate possible outcomes. The decision tree was constructed following the algorithm described by Breiman et al. (1984) and the methodological approach outlined by Gelman and Hill (2006), *Data Analysis Using Regression and Multilevel/Hierarchical Models* (Cambridge University Press). The final decision tree was visualised using functions developed by Wickham (2016) and Kassambara (2023).

The following main abbreviations are used throughout the manuscript: Diam – stem diameter of the host tree (cm); Nmb – number of mistletoe individuals (clusters); Def – defoliation (%); Degr – degree of defoliation.

Results

Empirical results and distribution patterns

Measurements were taken from 1158 *P. × canadensis* individuals. The Diam distribution of host trees was as follows: 36.8 % (10–19 cm), 39.1 % (20–29 cm), 17.9 % (30–39 cm), 5.5 % (40–49 cm), 0.7 % (>50 cm). Nearly half of the trees (49.8 %) were not infected by mistletoe. Distribution of remaining trees by Nmb was: 15.5 % (1 cluster), 16.7 % (2–4 clusters), 10.0 % (5–9), 6.3 % (10–19), 1.6 % (20–29), and 0.2 % (>30).

According to the classification of

Whitaker et al. (2025), the distribution of the examined host trees was as follows: 49.8 % – no visible infestation; 42.2 % – slightly infested; 7.9 % – moderately infested; and 0.2 % – heavily infested.

The distribution of Canadian poplar host trees by defoliation levels was as follows: 47.4 % – no damage, 21.4 % – mild, 23.1 % – moderate, and 8.1 % – severe. A few dead trees were found but excluded due to glyphosate treatment.

Statistical analysis and correlation modelling

Correlation analysis (Fig. 2A) showed that all indicators were statistically significant. Notably, the relationship between Nmb and Def and between Nmb and Diam was much stronger than that between diameter and defoliation.

Since nearly half of the measured trees in the study area were free of mistletoe, these were excluded and the correlation coefficients recalculated to assess their influence on the results (Fig. 2B). Excluding these trees did not alter the statistical interpretation.

Regression lines (Fig. 3) show no cor-

relation between defoliation and diameter in Group A (no or one mistletoe plant), but a strong, significant correlation in Group B (more than one mistletoe individual). The boxplot diagram in the same figure illustrates this pattern.

Therefore, Def does not change with Diam while in the presence of mistletoe it increases with Diam. The influence of the number of Nmb on Def is illustrated in the boxplot diagram (Fig. 4).

ANOVA statistical analysis (Table 1) confirms that the number of mistletoe clusters significantly affects defoliation.

Post-hoc Tukey's HSD test (Table 2) shows that even two mistletoe clusters cause defoliation, which is statistically more significant compared to zero number of clusters.

Defoliation with two or more mistletoe clusters was statistically significantly greater than that with '0' clusters in terms of their average values. If '0' clusters are taken as a control condition, and the remaining measurements as an infected group, then according to Dunnett's criterion, even the presence of one mistletoe affects the average values of defoliation, although less pronounced (Table 3).

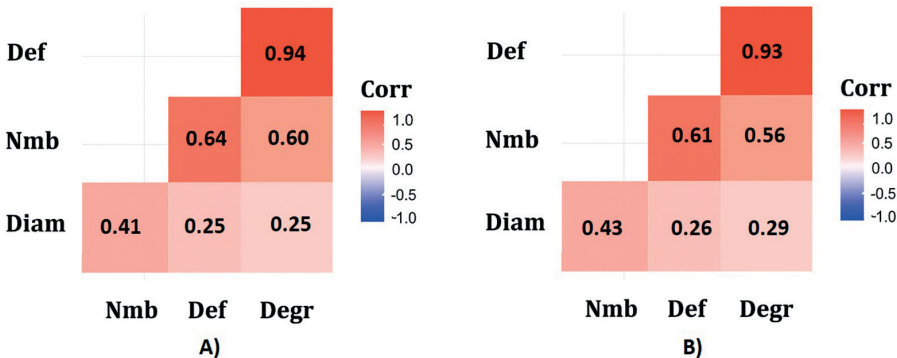


Fig. 2. Correlation coefficients between the studied indicators: A) with complete data; B) without data from measurements of trees that do not have mistletoe (Nmb = 0).

Note: Def – defoliation, %; Nmb – number of mistletoe individuals per host; Diam – stem diameter of the host tree; Degr – degree of defoliation.

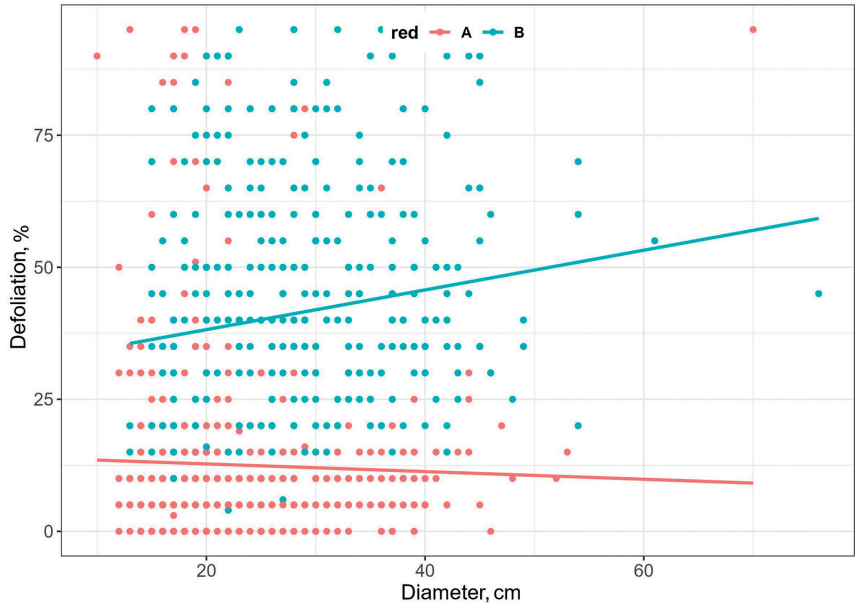


Fig. 3. Regression lines illustrating the relationship between defoliation, diameter, and number of mistletoe clusters for Group A (trees with no or a single mistletoe cluster, $N \leq 1$) and Group B (trees with more than one cluster, $N > 1$).

Note: In Group A, defoliation is not significantly related to diameter ($p = 0.3687$), whereas in Group B this relationship is positive and statistically significant ($p = 0.00135^{**}$).

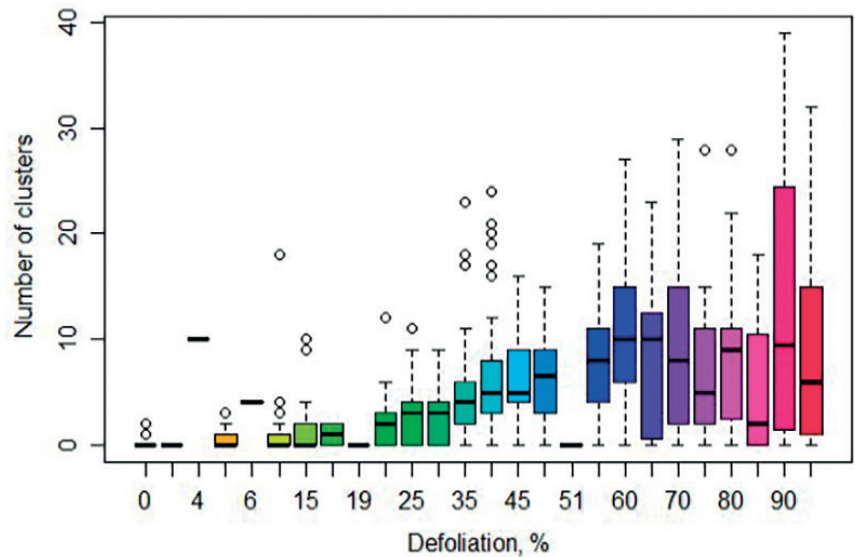


Fig. 4. Boxplot diagram showing changes in defoliation relative to the number of mistletoe individuals per tree.

Table 1. ANOVA-table for the influence of the number of mistletoe clusters on defoliation.

Source of variation	Df	Sum Sq	Mean Sq	F value	p (Pr>F)
Number of clusters	29	155,868	5375	16.98	$p < 2e-16$ ***
Residuals	551	174,401	317		

Note: *** indicates statistical significance at $p < 0.001$.

Table 2. Comparison of mean defoliation values using Tukey's HSD test.

Designation of differences	Estimation	Lower limit	Upper limit	p adj
2-0	11.6867	3.3978	19.9755	5.5e-05
3-0	18.6150	10.5486	26.6814	1.3e-13

Note: 2-0 – the difference in defoliation between the group with two mistletoe individuals (2 clusters) and the group without mistletoe (0 clusters); 3-0 – the difference in defoliation between the group with three mistletoe individuals (3 clusters) and the group without mistletoe (0 clusters).

Table 3. Comparison of mean defoliation values by Dunnett's test.

Designation of differences	Estimation	St. error	t-value	p (Pr > t)
1-0	4.641	1.426	3.255	$p = 0.034401$ *
2-0	11.687	2.196	5.322	$p < 1e-05$ ***

Note: 0, 1 and 2 are the average values for the number of mistletoe clusters; 1-0 – the difference in defoliation between the group with one mistletoe individual (1 cluster) and the group without mistletoe (0 clusters); 2-0 – the difference in defoliation between the group with two mistletoe individuals (2 clusters) and the group without mistletoe (0 clusters); * indicates $p < 0.05$; *** indicates $p < 0.001$.

The decision tree algorithm (Fig. 5) was applied to classify trees according to crown defoliation as a function of the number of mistletoe clusters. Each node represents a threshold of mistletoe abundance that optimally separates the data into groups with distinct defoliation levels.

Each node summarises the composition of the subset of trees that falls into it after applying the splitting criteria based on mistletoe abundance (Nmb). The percentages indicate how many trees in that subgroup exhibit a given crown-defoliation level, and together these percentages sum to 100 %. For example, a node label such as NoD = 54 %, LowD = 33 %, MedD = 11 %, StrD = 2 % means that within this subset of trees (defined by the preceding thresholds of mistletoe clusters), 54 %

show no visible defoliation, 33 % have mild defoliation, 11 % moderate, and 2 % severe crown loss.

Thus, the numeric values in each coloured label quantify the relative frequency (probability) of each defoliation class at that decision-tree node. Interpreting them from left to right along the branches allows identification of how increasing mistletoe abundance shifts the distribution of trees from healthy (NoD/LowD) toward more severely defoliated categories (MedD/StrD/Dead).

The initial split occurs at Nmb = 1, separating uninfected or lightly infected trees from those with higher infestation. The next major division is at Nmb = 4, beyond which the probability of moderate to severe defoliation sharply increases. This

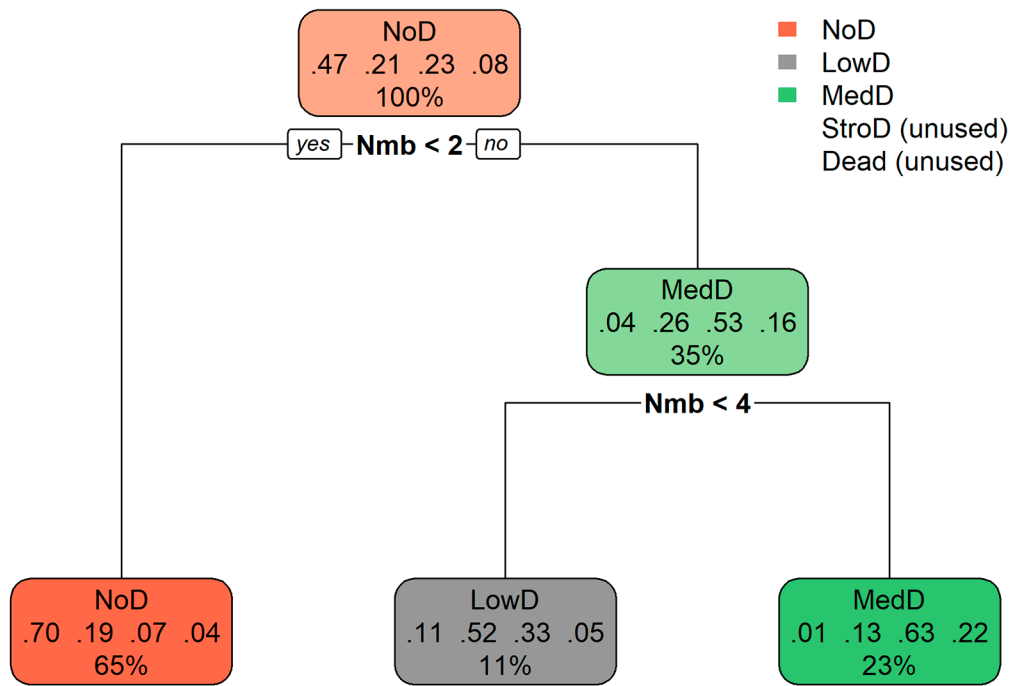


Fig. 5. Decision tree for classifying defoliation based on the number of mistletoe clusters.
Note: NoD – no defoliation ($\leq 10\%$); LowD – mild ($10\text{--}25\%$); MedD – moderate ($25\text{--}60\%$); StroD – severe ($60\text{--}99\%$); Dead – completely defoliated (100%). Numbers in the coloured labels indicate the proportion of trees (in %) belonging to each defoliation class within the respective decision-tree node.

hierarchical structure allows the identification of quantitative thresholds of mistletoe presence, corresponding to distinct crown-condition categories. Such a visual model provides an intuitive and transparent means of interpreting the interaction between infestation intensity and tree vitality, linking the number of mistletoe clusters directly to measurable changes in crown status.

Figure 6 illustrates how these thresholds correspond to mean values of stem diameter, defoliation level, and infestation intensity within the respective nodes. The general trend demonstrates that with increasing mistletoe abundance, both defoliation percentage and its severity class

increase proportionally.

Discussion

Currently, mistletoe infestation in the study area does not cause complete tree mortality, likely due to the low infestation levels. Most infected trees fall into the ‘low’ category per Whitaker et al. (2025). Only two host trees had high level of infestation. A bit less than 50% of the trees showed no defoliation, which is close to the percentage of uninfected trees.

In addition to mistletoe infestation, the phytosanitary status of the examined poplar plantations is also influenced by

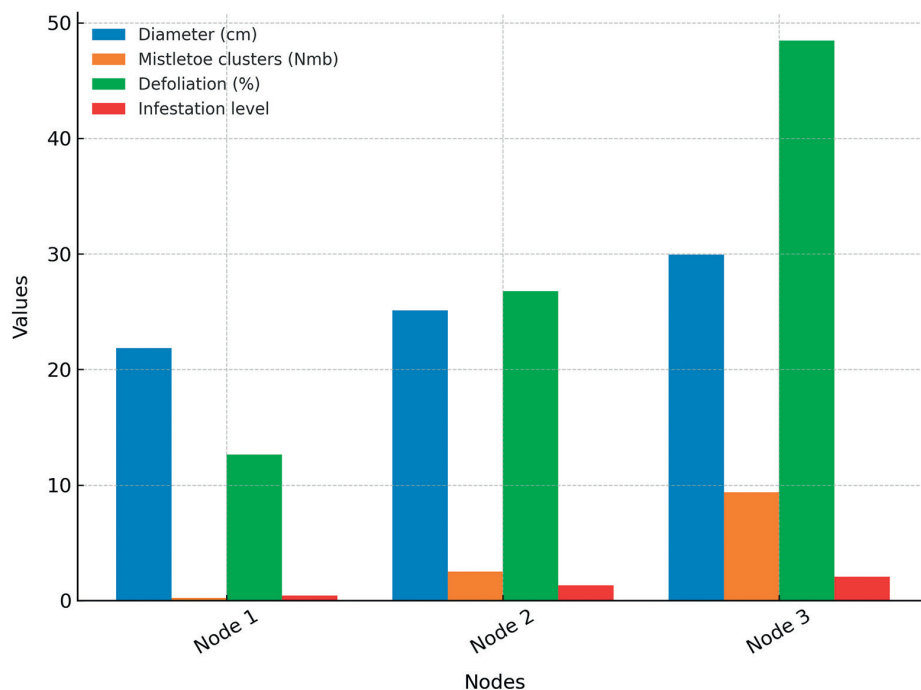


Fig. 6. Mean values of the four studied parameters in the nodes of the decision tree.

the natural ageing of stands, which in our case are between 20- and 40-year-old. With increasing age, processes, such as heartwood decay, wood rotting, and bark necrosis, can intensify, leading to progressive deterioration of crown vitality and contributing to higher levels of defoliation, independent of mistletoe infestation. In Bulgarian poplar plantations, bark necroses caused by fungal pathogens of the genera *Cytospora*, *Phomopsis*, *Fusarium*, *Dothichiza*, and others are widespread and may act synergistically with abiotic stresses, such as drought, poor water balance, or mechanical injuries (Bencheva and Belyova 2017). These findings underline that crown defoliation in poplars is the cumulative result of multiple interacting factors, where mistletoe represents one of several stressors that accelerate the decline in tree health.

Among infected trees, low and moderate defoliation dominates. Defoliation below 25 % is not considered a negative process, but >25 % indicates deteriorating health (Eichhorn et al. 2016). Trees with diameters <30 cm dominate the area. Older trees attract more birds and this is one of the main reasons for the greater number of mistletoe clusters established on them.

The role of avian dispersal is particularly pronounced, as the study site lies along the Via Pontica flyway – a major migration corridor where overwintering thrushes, such as Mistle thrush (*Turdus viscivorus* Linnaeus, 1758), Fieldfare (*T. pilaris* Linnaeus, 1758), Song thrush (*T. philomelos* Brehm, 1827), Redwing (*T. iliacus* Linnaeus, 1758) and Blackbird (*T. merula* Linnaeus, 1758), are common (Iankov 2007, Bulgarian Society for the

Protection of Birds 2020). These species are known as dispersers of *Viscum album* berries (Watson 2004), and their winter foraging activity likely facilitates mistletoe seed deposition on poplar crowns along the Black Sea coast.

The observed defoliation patterns are similar to those in Whitaker et al. (2025), where mistletoe influences defoliation and depends on the diameter, respectively the age of the tree. According to the same author, environmental factors, particularly low soil moisture and high light availability, exacerbated the severity of mistletoe-induced defoliation. These findings suggest that mistletoe proliferation poses a considerable threat to tree health in temperate forests, particularly under conditions of abiotic stress.

According to Kartawik et al. (2023), mistletoe's enduring and comprehensive systemic effects transform the pine host from modular to unitary in terms of space and time. This metabolic alteration instigated by mistletoe can introduce a new metabolic identity for the host, enhancing the complexity and diversity of the forest canopy. This initiates a chain of ecological consequences detrimental to pine herbivores. Dobbertin and Rigling (2006) conclude that mistletoe infection can be considered as both a predisposing factor for tree death, by increasing needle loss following drought and a contributing factor by increasing water stress during drought.

Given their current age structure, declining vitality, and susceptibility to mistletoe infestation and other stressors, the long-term prospects for these plantations are limited. Natura 2000 management planning may thus favour the gradual replacement of aging poplar stands with the restoration of natural dune ecosystems as a more sustainable conservation strategy.

The use of the decision tree allows

for more precise threshold values for the presence of mistletoe in the process of infestation. The influence of two mistletoe individuals on defoliation is stronger in smaller-diameter or submature trees (Diam $\leq 14\text{--}15$ cm), which are still in the phase of active radial growth and crown expansion. When more than four mistletoe clusters are detected on a single host, their influence on defoliation becomes noticeable also in larger-diameter, mature trees (Diam > 30 cm), as such infestation levels create a risk of further increase in mistletoe abundance within the same crown not only through zoochorous, but also through gravitational seed dispersal (Glogov and Bogdanov 2024).

Beyond its descriptive role, the decision tree also provides a practical forecasting framework. Because mistletoe clusters remain visible during the leafless winter period, the model can be used to estimate the likely degree of crown defoliation even when foliage is absent. This predictive capability enables foresters to assess tree vitality and make management decisions during the dormant season – such as prioritising individuals for removal, monitoring, or treatment – based solely on the visible number of mistletoe clusters. In this sense, the decision tree functions as a rapid, field-applicable tool for early detection of declining trees and for planning selective sanitation or conservation measures in dune plantations affected by *Viscum album*.

The results from the present study are also consistent with broader European findings demonstrating the context-dependent nature of *Viscum album* impact. For instance, Camarero et al. (2019) showed that mistletoe exacerbates the effects of other stressors, such as insect defoliation and drought, leading to long-term growth suppression detectable in

wood anatomy and water-use efficiency. Similarly, Mutlu et al. (2016) confirmed that mistletoe-induced oxidative stress intensifies during summer droughts in *Pinus sylvestris*, emphasising the interactive rather than isolated role of parasitic load. The patterns observed in the Nessebar plantations thus correspond to these multi-stressor dynamics, where defoliation is not only a direct symptom of parasitism but also a manifestation of cumulative physiological strain.

Furthermore, the moderate infestation levels recorded here align with the early-stage expansion phase described by Skrypnik et al. (2020) for urban landscapes, where *Viscum album* colonisation initially remains patchy but may accelerate once mature hosts become dominant in the canopy. This observation underscores the importance of early monitoring and adaptive management – principles emphasized by Bussotti et al. (2024) as central to forest-health assessment under climate change conditions. Integrating decision-tree models into such frameworks could therefore enhance the predictive capacity of local monitoring networks and contribute to more efficient management of coastal protective plantations in Natura 2000 dune habitats.

Conclusions

A positive correlation was confirmed between mistletoe individuals per host tree and both trunk diameter and crown defoliation.

At present, mistletoe infestation in the study area does not directly cause total tree death, but it may enhance other factors (abiotic and biotic) that do.

Based on the developed decision tree, up-to-date models can be created for the

specific region predicting the degree of defoliation (and the related changes in the health of poplars) based on data regarding the diameter of the host stem and the quantitative participation of mistletoe.

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Response of catalase and cellulase to microbial biomass carbon and some soil indicators in forest, agricultural and urban soils of Sofia Municipality with and without influence of local water sources

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Thematic field: Ecosystem biodiversity, conservation and environmental restoration

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Abstract

Microbial biomass carbon (MBC) and the enzymatic activities of catalase and cellulase were investigated in soils within Sofia Municipality in an agricultural ecosystem (Vrazhdebna district), forest ecosystems (Vitosha Nature Park, Zheleznitsa, and Dolni Pasarel areas), and urban soil (green area, Dolni Pasarel village), in a seasonal context, with and without the influence of local water sources. MBC and the activities of catalase and cellulase were higher in the agricultural soils than in the forest soils, which is also attributable to the fact that they are tilled and irrigated, with trends of higher microbiological activity in spring compared to summer. In most soils of Vitosha, the inflow of water from local sources creates anaerobic conditions that reduce the accumulation of microbially derived carbon and the enzymatic activity of microorganisms. Soils in Vitosha, Zheleznitsa and Dolni Pasarel are acidic, while those in Vrazhdebna are neutral, suggesting mainly bacterial biomass in the latter and more mould fungi in acidic soils; anaerobic conditions in waterlogged sites further limit mycelial microorganisms. Correlation analysis showed a strong negative relationship between MBC and soil moisture ($r \approx -0.73$; -0.69), a weak positive relationship with pH ($r \approx 0.08$ – 0.15), a moderate positive relationship with temperature ($r \approx 0.30$ – 0.38) and catalase ($r \approx 0.50$ – 0.60) and a strong positive relationship with cellulase ($r \approx 0.61$ – 0.66). Catalase activity was moderately negatively correlated with moisture ($r \approx -0.53$; -0.45) and moderately positively with pH ($r \approx 0.49$ – 0.52); cellulase showed a weak to moderate negative association with moisture ($r \approx -0.33$; -0.27), a weak to moderate positive association with temperature ($r \approx 0.28$ – 0.31) and a moderate negative association with pH ($r \approx -0.21$; -0.26). Regression analysis confirmed the role of pH as a stable predictor – positive for catalase and negative for cellulase in both seasons, and showed that in spring catalase was the strongest positive predictor of cellulase, whereas in summer cellulase was significantly influenced by temperature, MBC, depth and catalase.

Key words: agricultural soils, enzymatic activities, forest soils, microbial carbon, urban ecosystem.

Introduction

The accumulation of microbial biomass carbon in soils from forest and agroecosystems in urban environments, is of essential importance for the content of organic carbon in them and the course of the carbon cycle in nature. Organic carbon of microbial origin indicates the activity of soil microorganisms, which in turn are sensitive indicators of the processes taking place in soils. They participate in the cycle of substances, provide nutrition for plants and self-cleaning of soils from pollutants. The activity of soil microorganisms is also indicative through the study of the enzymes produced by them. Cellulase is a primary enzyme involved in the carbon cycle in the decomposition of cellulose, a main component in plant residues. Catalase is a respiratory enzyme that participates in the decomposition of toxic hydrogen peroxide into water and oxygen. All three indicators – microbial biomass carbon, cellulase and catalase activity, are used to study microbiological soil activity. However, results are often contradictory due to the complex of environmental factors affecting the quantity, composition, and activity of soil microflora.

Soil microbial biomass is the living mass of organic matter, half of which is located in the soil top layer (15 cm) (Das et al. 2023) and represents a labile pool of nutrients, including bacteria, actinomycetes, fungi, algae, protozoa, and other soil microfauna (Henrot and Robertson 1994), playing a major role in the decomposition and build-up of the soil carbon stock (Das et al. 2023). According to a study (Das et al. 2023) covering publications on microbial biomass carbon from 1989 to 2022, microbial organic carbon stocks in soils of different forest ecosystems worldwide ranged from 55 mg·kg⁻¹ (decid-

uous forests) to 2214.4 mg·kg⁻¹ in montane mixed forests. Total organic carbon stocks ranged from 800 mg·kg⁻¹ (boreal forests) to 350,800 mg·kg⁻¹ (mixed natural forests). Microbial carbon was positively correlated with organic carbon stocks and soil basal respiration and influenced by temperature, moisture, seasonal factors, and other environmental conditions. Soil microbial biomass includes microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass phosphorus, and microbial biomass sulphur, with MBC and MBN accounting for 1–5 % and 2–6 % of the total soil C and N content, respectively, and tending to be relatively more stable (Babur and Dindaroglu 2019). Approximately 1.4 % of the global soil organic C content is of microbial origin, but it contributes significantly to the global C cycle (Wardle 1992). The contribution of carbon from microbial biomass to total organic carbon in agricultural soils has been reported to be 1.13–3.37 %, and microclimatic variations as well as different agricultural practices have been found to influence changes in microbial biomass (Bhuyan et al. 2013).

The accumulation of microbial biomass and its decomposition with the help of produced enzymes depend on both the amount and activity of the soil microflora and on environmental factors: climate, mechanical composition and structure of the soil, nutrient availability, soil temperature and moisture, type of vegetation, also on the depth of sampling and other factors (Gulde et al. 2008, Russell et al. 2015, Hassink 2016, Kara et al. 2016, Magnússon et al. 2016, Babur 2019, Malcheva 2020a, Qu et al. 2023, Zhan 2024). The ratio of microbial biomass carbon to total organic carbon can be indicated as an indicator of the dynamics of carbon in the soil (Insam et al. 1989). Soil microbial diversi-

ty, microbial biomass, microbial respiration and soil enzymes should be monitored as more indicative of any changes in soil conditions to provide more accurate and rapid information on soil health and quality and to measure ecosystem health (Rice et al. 1986, Smith 1993, Sparling 1997, Nielsen and Winding 2002, Alvarez et al. 2009, Schnecker et al. 2023, Zhang 2024).

Cellulase is a key enzyme in the carbon cycle and, together with catalase, is a sensitive indicator that determines the biochemical characteristics of soils (Torres et al. 2016, Malcheva et al. 2023). Soil biogenicity and cellulase activity are higher in *Fagus sylvatica* L. beds than in *Pinus sylvestris* L., decreasing with depth in the soil profile (Malcheva et al. 2024). Cellulase activity depends on soil temperature and moisture, organic matter content, amount and composition of microorganisms (bacteria dominate), sampling depth, soil type, mechanical composition, structure and pH of the soil, type of plant residues and other factors (Kanazawa and Miyashita 1987, Malcheva et al. 2015a, 2015b, 2019, 2024, Malcheva 2020b, 2020c, Naskova et al. 2016, Datta 2024). A very strong positive correlation has been found between catalase activity and microbial biomass in carbon, more pronounced in broadleaved than in coniferous forest vegetation. (Malcheva et al. 2023). Both MBC and catalase activity decrease sharply from dead forest litter to A soil horizon, followed by a weaker and smoother decrease with increasing soil depth. Catalase activity correlates with iron content, hygroscopic moisture, humus, the amount and activity of soil microorganisms, soil temperature and pH, soil type, clay content, sampling depth, vegetation type, presence of inhibitors, research methods, and agrotechnical practices (Grozeva and Nustorova 1995; Uzun and Uyanöz 2011; Malcheva

et al. 2015a,b, 2019; Naskova et al. 2016; Malcheva 2020b,c).

The aim of the present study was to analyse the seasonal (spring vs. summer) dynamics of sensitive microbiological and enzymatic indicators – MBC, catalase, and cellulase activities – in relation to selected soil parameters in agricultural, forest, and urban soils of Sofia Municipality, including irrigated soils and over-waterlogged soils near local water sources. We hypothesised that: (i) MBC, catalase, and cellulase activities would be significantly higher in agricultural soils compared to forest and urban soils due to cultivation and irrigation; (ii) over-moistening and partial anaerobiosis in soils near local water sources (springs, boreholes, fountains) would lead to lower MBC and enzyme activities; (iii) soil temperature and pH would emerge as significant predictors of MBC and enzymatic activities; and (iv) MBC and enzyme activities would be interdependent, showing significant positive associations in the regression models.

Materials and Methods

The analysed soils were collected from four regions within Sofia Municipality during two seasons – spring and summer: the villages of Zheleznitsa and Dolni Pasarel, the Vrazhdebna district, and Vitosha Nature Park. Sampling included soils influenced by local water sources (fountains, springs, or irrigation) as well as nearby soils without such influence. Soil samples were collected at two depths: 0–10 cm and 10–25 cm. According to the World Reference Base for Soil Resources (WRB 2022), the soils in Vitosha Nature Park and the Zheleznitsa area are predominantly brown forest soils (Cambisols), with exceptions at SS17 and SS18 (Regosols)

and SS21 (Fluvisols). The soils in Dolni Pasarel area are cinnamon forest soils, also classified as Cambisols. At locations directly influenced by water from fountains and springs, Cambisols with Stagnic properties (SS1, SS5, SS13, SS15, SS19), Regosols with Stagnic properties (SS17), and Cambisols with Gleyic properties (SS3) were identified. The soils in Vrazhdebna Training and Experimental

Field are alluvial-meadow soils (Fluvisols). Previous studies report that Cambisols are the prevailing soil type in Vitosha Nature Park (Karatoteva 2016, Hristov et al. 2025), while other authors indicate that the soils of the University of Forestry's Vrazhdebna Training and Experimental Field are Fluvisols (Kercheva et al. 2021, Dimitrov 2024). The sampling sites (SS) are listed and described in Table 1.

Table 1. Sampling sites and soil description.

Sampling sites	Location, GPS coordinates (WGS84)	Altitude, m	Type of influence on soil (water source / plant cover)
SS1	Zheleznitsa 42°32'25.476" N, 23°23'5.604" E	900	Influenced by mineral water from a borehole. <i>Plantago major</i> L., <i>Athyrium filix-femina</i> (L.) Roth., <i>Urtica dioica</i> L.
SS2	Zheleznitsa 42°32'25.476" N, 23°23'5.648" E	900	Near SS1, not influenced by mineral water from a borehole. <i>Plantago lanceolata</i> L., <i>Urtica dioica</i> .
SS3	Zheleznitsa 42°32'25.542" N, 23°23'5.868" E	905	Influenced by a natural spring water. Without vegetation.
SS4	Zheleznitsa 42°32'25.575" N, 23°23'5.802" E	905	Near SS3, not influenced by natural spring water <i>Urtica dioica</i> , <i>Lolium perenne</i> L., <i>Poa pratensis</i> L.
SS5	Dolni Pasarel 42°32'23.266" N, 23°29'40.776" E	710	Influenced by roadside fountain water. <i>Hedera helix</i> L., <i>Geranium robertianum</i> L.
SS6	Dolni Pasarel 42°32'23.266" N, 23°29'40.732" E	710	Near SS5, not influenced by roadside fountain water. <i>Geranium robertianum</i> , <i>Lolium perenne</i> .
SS7	Dolni Pasarel 42°32'23.435" N, 23°29'43.498" E	708	Next to the Colourful fountain, not influenced by the water from it. <i>Geranium robertianum</i> , <i>Taraxacum officinale</i> F.H.Wigg., <i>Sambucus nigra</i> L.
SS8	Vrazhdebna 42°42'26.001" N, 23°26'11.804" E	538	Irrigated by an irrigation basin. <i>Brassica oleracea</i> L. var. <i>capitata</i> L.
SS9	Vrazhdebna 42°42'26.065" N, 23°26'11.805" E	538	Irrigated by a borehole 1. <i>Brassica oleracea</i> var. <i>capitate</i> .
SS10	Vrazhdebna 42°42'25.968" N, 23°26'11.848" E	538	Irrigated by a borehole 2. <i>Capsicum annuum</i> L.

Sampling sites	Location, GPS coordinates (WGS84)	Altitude, m	Type of influence on soil (water source / plant cover)
SS11	Vrazhdebna 42°42'25.968" N, 23°26'11.936" E	538	Irrigated by an outdoor fountain. <i>Capsicum annuum</i> .
SS12	Vrazhdebna 42°42'25.871" N, 23°26'11.892" E	538	Unirrigated soil. Without vegetation.
SS13	Vitosha Nature Park, Vitosha tulip area 42°35'10.590" N, 23°18'26.257" E	1665	Influenced by fountain water. <i>Euphrasia officinalis</i> L., <i>Luzula multiflora</i> (Ehrh.) Lej.
SS14	Vitosha Nature Park, Vitosha tulip area 42°35'10.558" N, 23°18'26.345" E	1665	Near SS13, not influenced by fountain water. <i>Pinus sylvestris</i> L., <i>Luzula luzuloides</i> (Lam.) Dandy & Wilmott, <i>Euphrasia officinalis</i> .
SS15	Vitosha Nature Park 42°36'29.890" N, 23°17'51.280" E	1270	Influenced by roadside fountain water. <i>Fagus sylvatica</i> L., <i>Geranium robertianum</i> .
SS16	Vitosha Nature Park 42°36'30.071" N, 23°17'51.302" E	1270	Near SS15, not influenced by roadside fountain water. <i>Fagus sylvatica</i> , <i>Geranium robertianum</i> .
SS17	Vitosha Nature Park 42°38'41.280" N, 23°14'25.692" E	910	Influenced by Stone fountain water. <i>Plantago major</i> , <i>Deschampsia cespitosa</i> (L.) P.Beauv.
SS18	Vitosha Nature Park 42°38'41.313" N, 23°14'25.736" E	910	Near SS17, not influenced by Stone fountain water. <i>Carpinus betulus</i> L.
SS19	Vitosha Nature Park 42°36'59.148" N, 23°14'18.624" E	1401	Influenced by Pavlova fountain water. <i>Fagus sylvatica</i> , <i>Athyrium filix-femina</i> , <i>Geranium robertianum</i> .
SS20	Vitosha Nature Park 42°36'59.181" N, 23°14'18.580" E	1401	Near SS19, not influenced by Pavlova fountain water. <i>Fagus sylvatica</i> , <i>Athyrium filix-femina</i> (L.) Roth., <i>Geranium robertianum</i> .
SS21	Vitosha Nature Park, Golden bridges area 42°36'36.020" N, 23°14'19.987" E	1382	Influenced by Vladayska River water. Without vegetation.
SS22	Vitosha Nature Park, Golden bridges area 42°36'36.013" N, 23°14'19.987" E	1382	Near SS21, not influenced by Vladayska River water. <i>Angelica sylvestris</i> L., <i>Plantago lanceolata</i> , <i>Lolium perenne</i> .

All sampling sites were located within the administratively urbanised area of Sofia Municipality (including protected natural areas, rural settlements, and an urban

experimental field). For the purposes of this study, soils were grouped both by land use and by genesis: (i) forest soils from protected natural areas (Vitosha Nature

Park) and rural settlements (Zheleznitsa and Dolni Pasarel); (ii) agricultural soils from the University of Forestry's Vrazhdebna Training and Experimental Field (cultivated and irrigated Fluvisols); and (iii) urban soils from green areas within settlements (SS7 – Dolni Pasarel, near the Colourful fountain). This dual classification highlights both the fact that all sites are located within Sofia Municipality (an urbanised territory) and the differences in soil origin and land use.

The sampling site location map was prepared using Google Earth Pro (version 7.3.6; Google LLC) (Fig. 1).

Sampling was performed under sterile conditions, as part of the samples were also analysed for major groups of micro-organisms. The samples were transported in a cooler at 4 °C, and analyses were initiated immediately upon delivery to the laboratory on the day of sampling. MBC was determined using the fumigation method (Cai et al. 2011). Soil extracts were prepared with potassium sulphate,

followed by digestion with a dichromate–sulphuric acid solution. The samples were heated at 140 °C for 30 min, and absorbance was measured spectrophotometrically at 350 nm. Carbon concentration was calculated using a glucose calibration curve.

Catalase activity was determined using the manganometric titration method (Khaziev 1976). In the experimental samples, hydrogen peroxide was added to the soil, while sulphuric acid was used for the controls. After 15 min of shaking on a shuttle apparatus, the reagents were exchanged, and shaking was continued for a further 15 min. The suspensions were then filtered and titrated with potassium permanganate until a pink colour appeared. Catalase activity was calculated as the difference between the volume of oxygen required for titration of the control and that of the experimental sample.

Cellulase activity was determined dynamically by placing sterile filter strips on the soils, periodically moistening and



Fig. 1. Sampling site location map.

counting the percentage of degraded area every 15 days with a grid standard (Khaziev 1976).

Soil temperature was monitored in the field using a temperature probe (model Sv218) at the time of soil sampling for microbiological and enzymatic analyses, once per season (spring and summer) at two depths: 0–10 cm and 10–25 cm.

Soil moisture was determined gravimetrically in the laboratory using a moisture balance (model DBS 60-3) by drying the samples at 105 °C until constant weight. Measurements were taken once per season (spring and summer) at two depths (0–10 cm and 10–25 cm) immediately after soil collection for microbiological and enzymatic analyses. Soil moisture was also monitored in the field using a moisture probe (model Sv218); results from both methods were comparable and averaged. Values of microbial biomass

carbon and enzymatic activities were subsequently expressed on an oven-dry soil basis using the determined moisture coefficients.

The active reaction (pH) of the soil was determined potentiometrically in a water extract according to ISO 10390:2005.

Data preprocessing and descriptive statistics were performed using Microsoft Excel 2010, while stepwise multiple regression analysis was conducted with IBM SPSS Statistics, version 25.0.

Results and Discussion

The accumulation of organic carbon of microbial origin was studied in two seasons – spring and summer at two depths (0–10 cm, 10–25 cm) and is presented in figures 2–5.

The values of MBC are higher in ag-

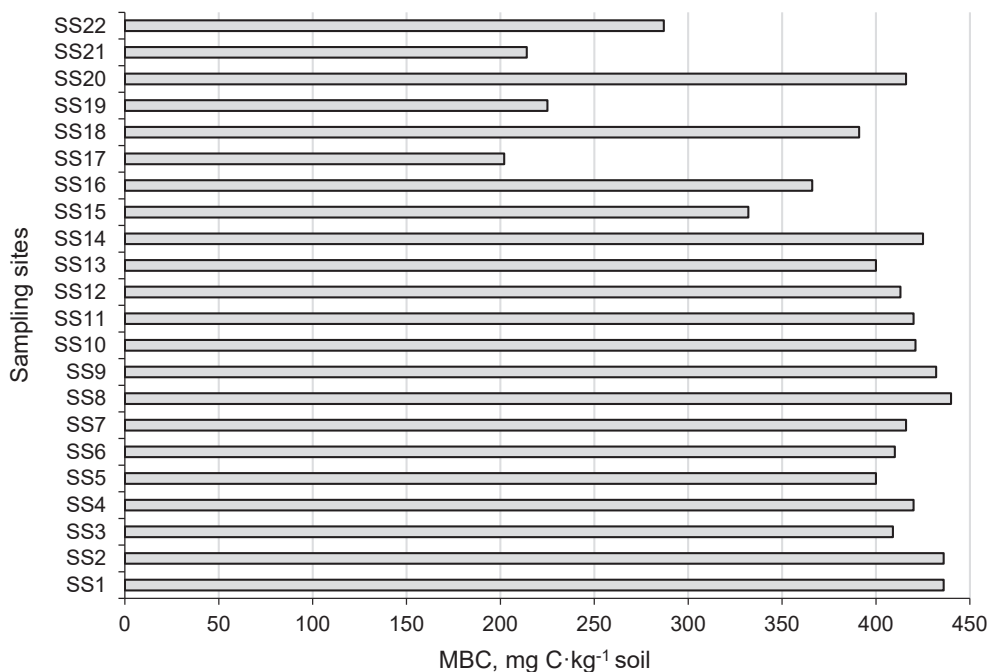


Fig. 2. Microbial biomass carbon in spring (0–10 cm).

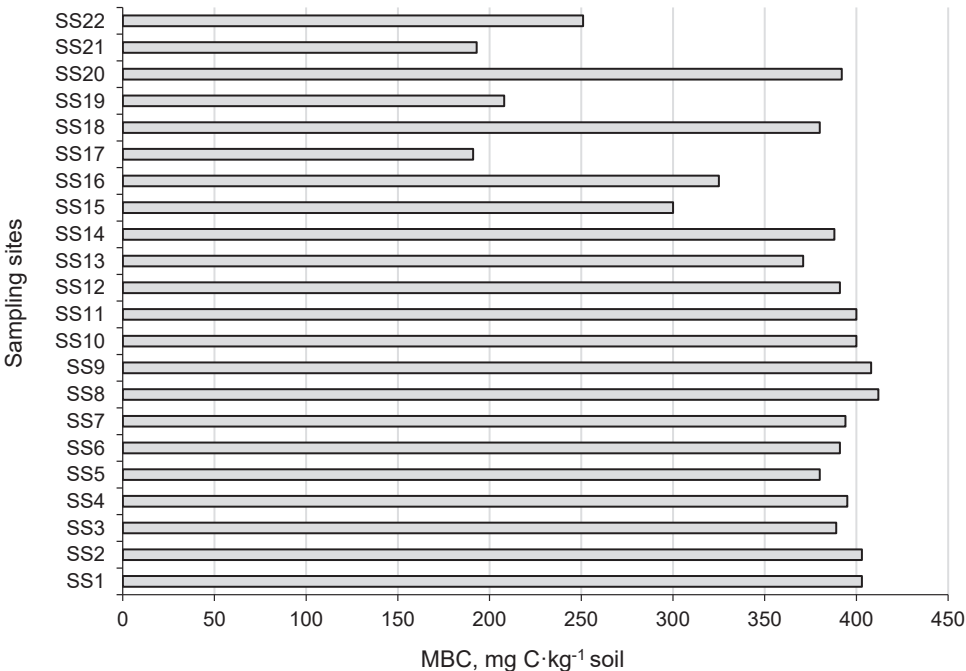


Fig. 3. Microbial biomass carbon in spring (10–25 cm).

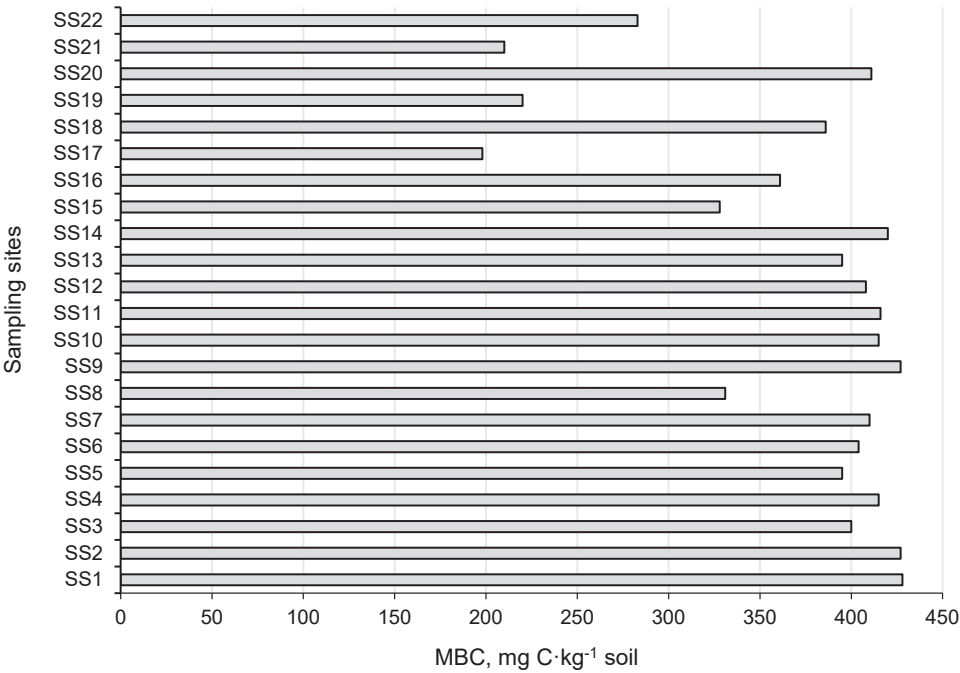


Fig. 4. Microbial biomass carbon in summer (0–10 cm).

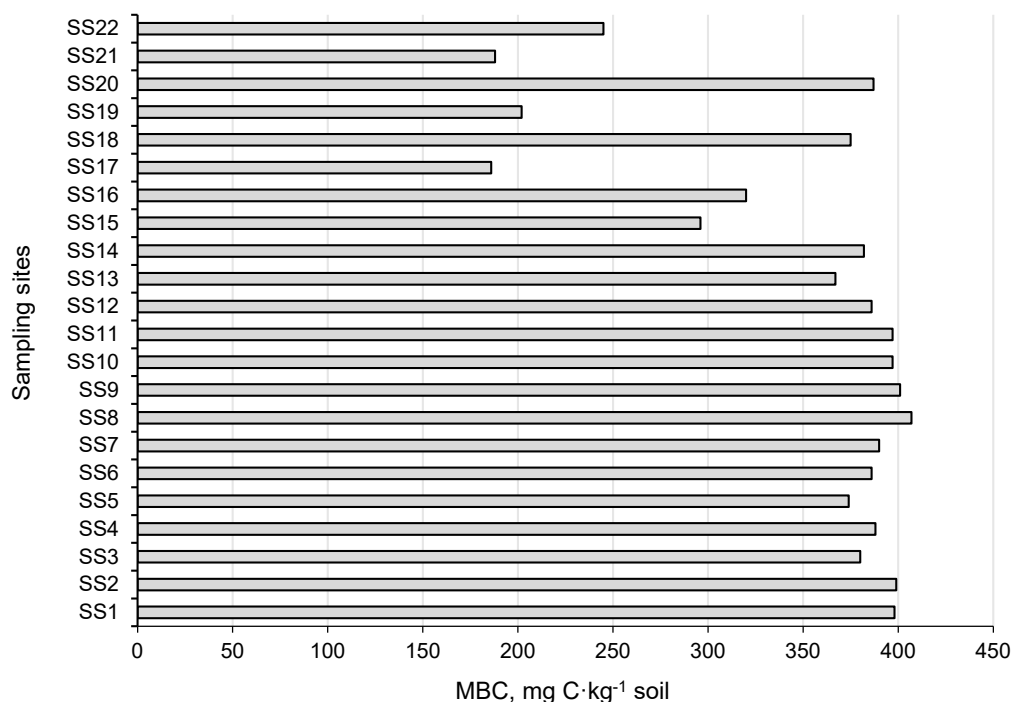


Fig. 5. Microbial biomass carbon in summer (10–25 cm).

gricultural soils compared to forest soils, which is also due to the fact that they are cultivated and irrigated. An increased accumulation of organic carbon is found in spring compared to summer. This trend depends on the quantity, composition and activity of soil microorganisms. Malcheva et al. (2025) found for the same variants that the total quantity of microorganisms is also higher in agricultural soils, with the main share in the composition of the soil microflora occupied by non-spore-forming bacteria, followed by bacilli, weaker and even absent development of mycelial groups of microorganisms – actinomycetes and mould fungi in waterlogged soils, due to the anaerobic conditions created. The same authors point out that the mineralisation rate of decomposition of organic substances in soils, however, does not

depend solely on the quantity of microorganisms. MBC depends on a complex of factors (CF), including the quantity, composition and activity of the soil microflora, soil temperature, moisture and pH, soil depth, soil type and mechanical composition, nutrient availability, the presence and type of vegetation, altitude and other site-specific conditions (Gulde et al. 2008, Russell et al. 2015, Hassink 2016, Kara et al. 2016, Magnússon et al. 2016, Babur 2019, Qu et al. 2023, Zhan 2024).

In spring, the MBC values in both soil layers are highest in agricultural soils from Vrazhdebna (SS8, SS9) and from Zheleznitsa, soils influenced by a mineral spring and in its immediate vicinity (SS1, SS2). These results correlate with a higher abundance of microorganisms and higher mineralisation activity in these soils

(Malcheva et al. 2025). The agricultural soils in Vrazhdebna are arable and regularly irrigated, while the mineral spring in Zheleznitsa is rich in dissolved organic matter, both contributing to enhanced microbial biomass. In summer, microbial organic carbon again reached its maximum values in the same soil samples, except for SS8, where MBC decreased more sharply than the total microbial abundance and mineralisation activity. The lowest MBC values were recorded in SS17, SS21, SS19 and SS22, corresponding mainly to Vitosha Nature Park sites, where high soil moisture and overmoistening from adjacent water bodies lead to leaching of the upper soil horizon and a reduction in organic matter and microbial abundance. Seasonal variations in temperature, precipitation, plant growth and the accumulation of organic matter from different types of plant residues also have a major impact on soil microbial biomass (Maithani et al. 1996, Chen et al. 2005, Tonon et al. 2005, Devi and Yadava 2006, Schneckner et al. 2023, Qu et al. 2023). Maximum microbial biomass has been reported either in spring (Diaz-Raviña et al. 1995, Zhu and Carreiro 2004), in summer (Gallardo and Schlesinger 1994, Zhong and Makechin 2006), or without pronounced seasonal trends (Bauhus and Barthel 1995). The studied sites are part of an urban environment, with soils variably affected by transport traffic emissions (mainly in Vrazhdebna) and tourism or mineral extraction (mainly in Vitosha Natural Park and Zheleznitsa). In soils under greater anthropogenic influence, organic matter undergoes more intense mineralisation and carbon loss to the atmosphere, limiting humus accumulation, whereas in urban park areas, elevated soil organic carbon values are often observed (Nikolov et al. 2020). In our study, however, the park

sites were strongly affected by topsoil erosion due to inflow from nearby water bodies. Disturbance of soil texture, increase bulk density and reduced soil moisture are main factors responsible for the depletion of microbial biomass in urban soils (Rai et al. 2018).

The studied enzymes – catalase and cellulase – reached their maximum activities in spring and showed higher values in agricultural soils. Catalase activity correlated with microbial biomass accumulation: the highest enzyme levels were recorded in soils from Vrazhdebna (SS8 and SS9) and in the site influenced by the mineral spring in Zheleznitsa (SS1). The lowest catalase activities were recorded in soils near Vladayska River – Golden Bridges (SS21 and SS22) and at Pavlova fountain (SS19), while comparatively low values were also observed in soils from the other sites located within Vitosha Nature Park. It should be noted that catalase, in addition to its microbial origin, may also have a plant origin. Regarding cellulase activity, complete cellulose degradation was detected at one of Vrazhdebna sites (SS8, both depths, spring) and in the soil influenced by the mineral spring in Zheleznitsa (SS1, both depths, spring). Catalase activity is shown in figures 6–9.

Similarly, catalase activity is influenced by the same CF, with additional effects related to humus, iron content, clay fraction, and the presence of inhibitors, research methods, agrotechnical measures (Grozeva and Nustorova 1995; Uzun and Uyanöz 2011; Malcheva et al. 2015b, 2019, 2023; Naskova et al. 2016; Malcheva 2020b,c; Wang et al. 2024). The absence of vegetation at sites directly affected by water sources, together with washout by surface flow, also negatively impacts microbial biomass accumulation and enzyme activities.

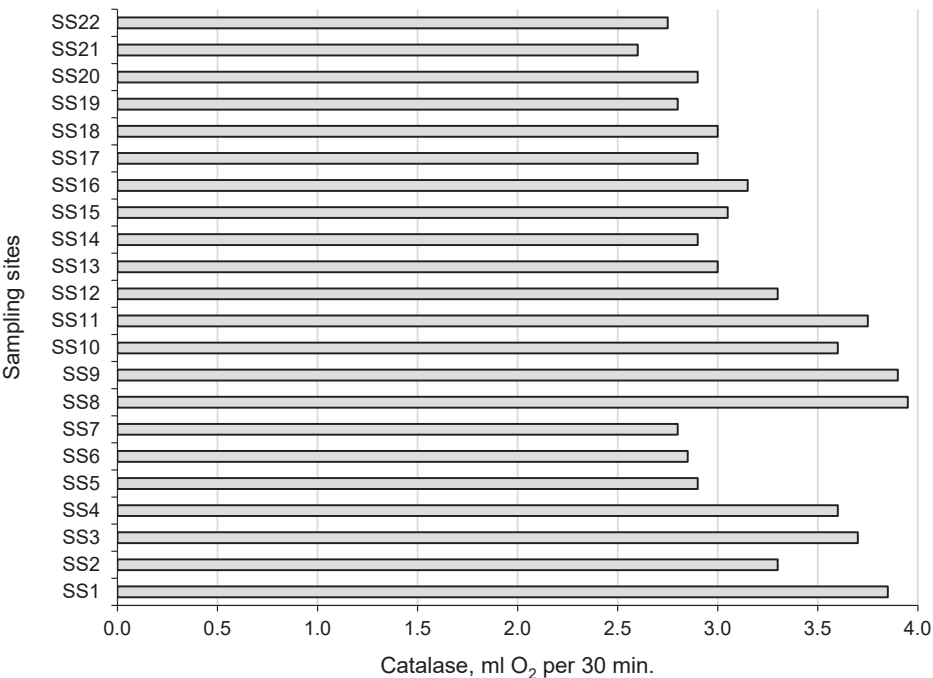


Fig. 6. Catalase activity of soil microorganisms in spring (0–10 cm).

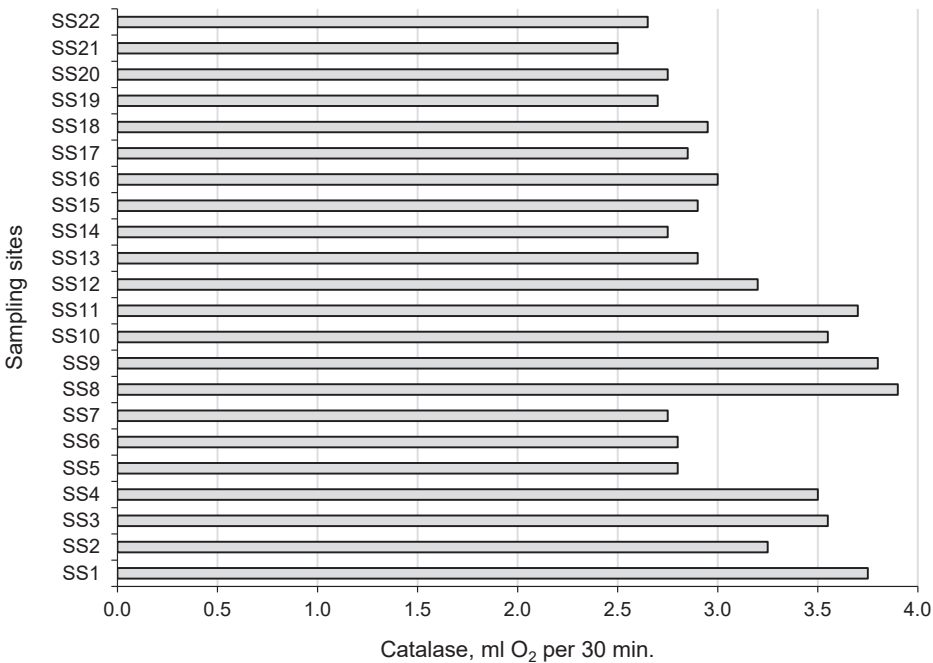


Fig. 7. Catalase activity of soil microorganisms in spring (10–25 cm).

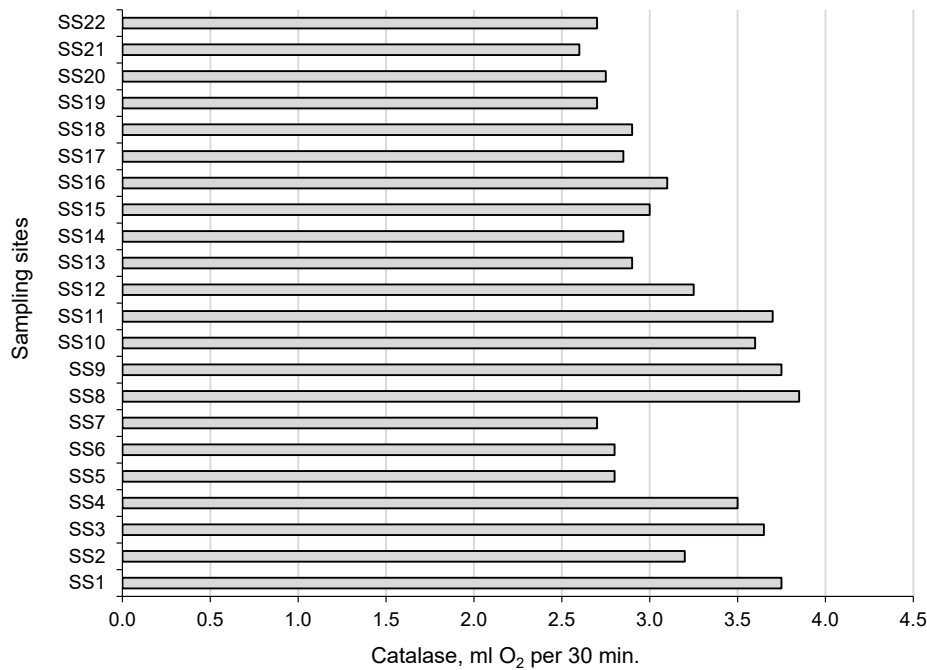


Fig. 8. Catalase activity of soil microorganisms in summer (0–10 cm).

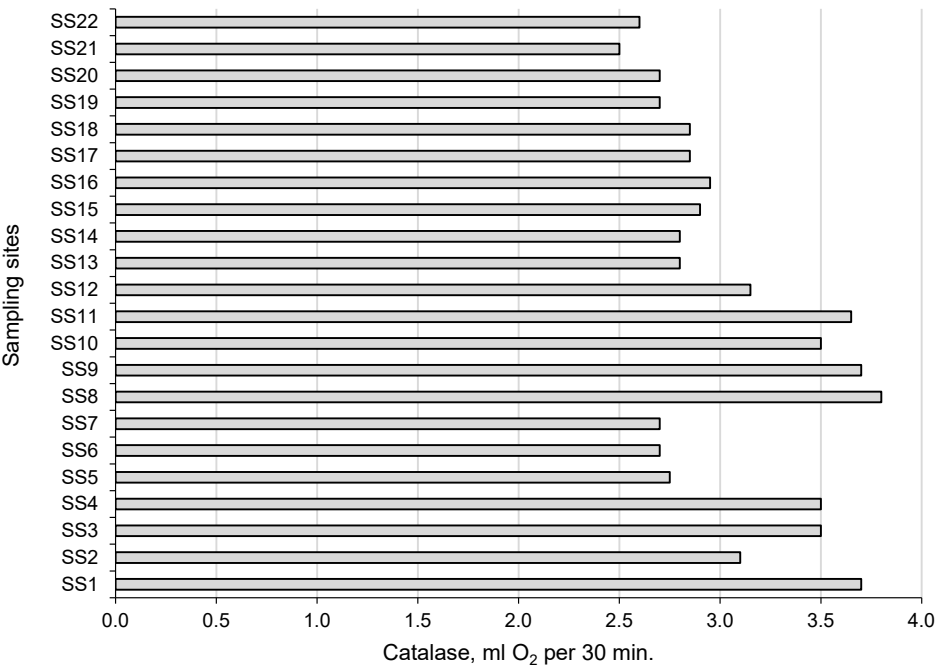


Fig. 9. Catalase activity of soil microorganisms in summer (10–25 cm).

Cellulase activity is significantly lower at SS17 (soil influenced by the water flow of Stone fountain in Vitosha Nature Park) – 4 times in spring and 3 times in summer lower compared to the highest values. This trend is maintained in both seasons (figs 10–13).

Cellulase activity is also determined by the same CF that influence microbial biomass and catalase activity, with the abundance and composition of microorganisms – particularly bacteria – playing a decisive role in the decomposition of plant residues and the overall cellulolytic activity (Kanazawa and Miyashita 1987, Malcheva et al. 2015a, 2015b, 2019, 2024, Naskova et al. 2016, Malcheva 2020b, 2020c, Datta 2024).

In summer, the accumulation of microbial biomass carbon and the production of enzymes is by mesophilic groups of microorganisms, while in spring, psy-

chrophiles also participate, especially in forest areas with higher altitudes. In most of the waterlogged soils of Vitosha Nature Park, due to spillage of water from local water sources to them, the abundance of aerobic microorganisms decreases at the expense of anaerobes (Malcheva et al. 2025), the accumulation of microbial biomass decreases and, accordingly, the enzymatic activity of microorganisms decreases. The values of soil temperature and moisture in spring and summer are presented in figures 14 and 15.

The dynamics in the temperature regime of the soils is more significant in spring – higher temperatures in more open areas (Vrazhdebna, Zheleznitsa and Dolni Pasarel) and lower in the sites of Vitosha Nature Park. For soil moisture, the trends are opposite – higher values in the soils of Vitosha Nature Park and the soil

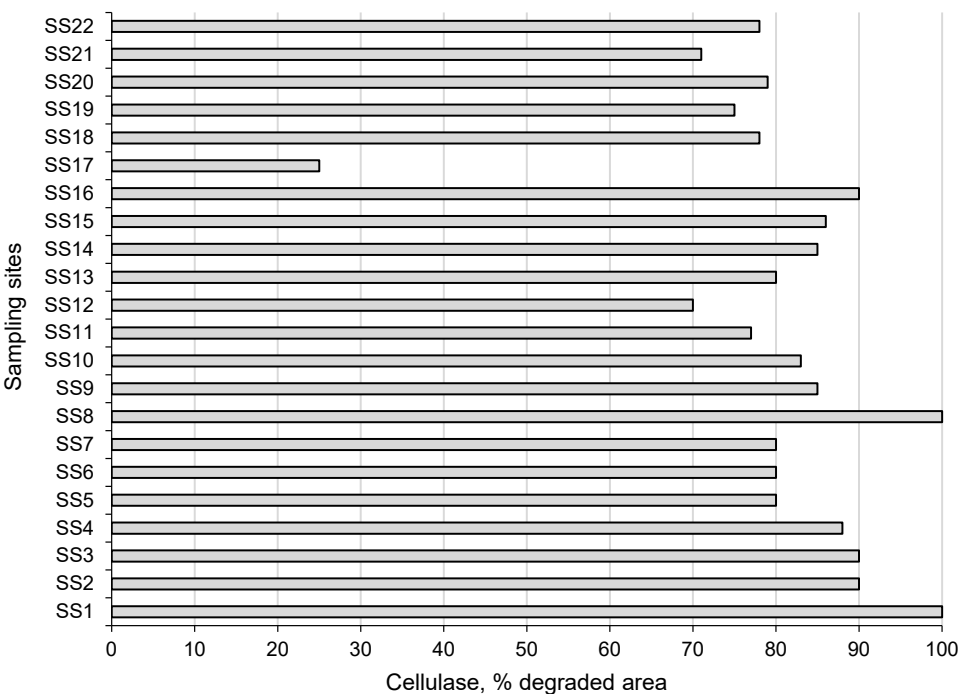


Fig. 10. Cellulase activity of soil microorganisms in spring (0–10 cm).

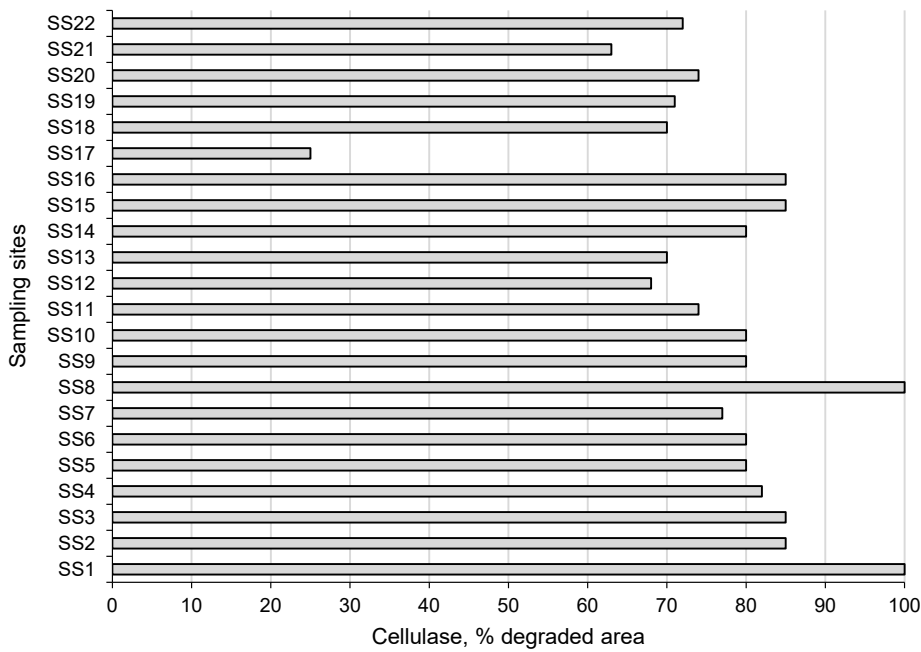


Fig. 11. Cellulase activity of soil microorganisms in spring (10–25 cm).

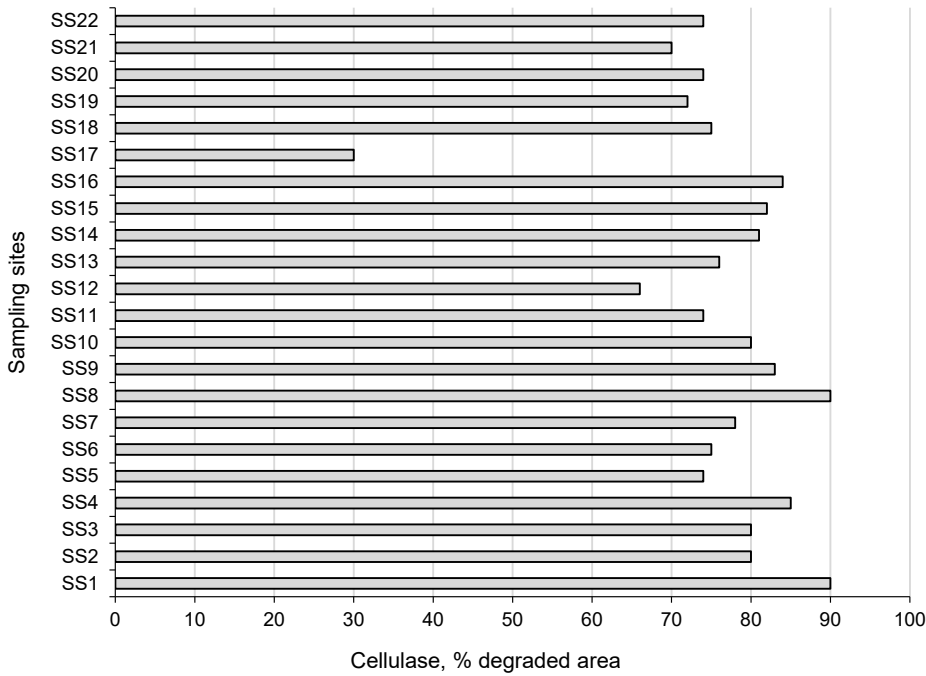


Fig. 12. Cellulase activity of soil microorganisms in summer (0–10 cm).

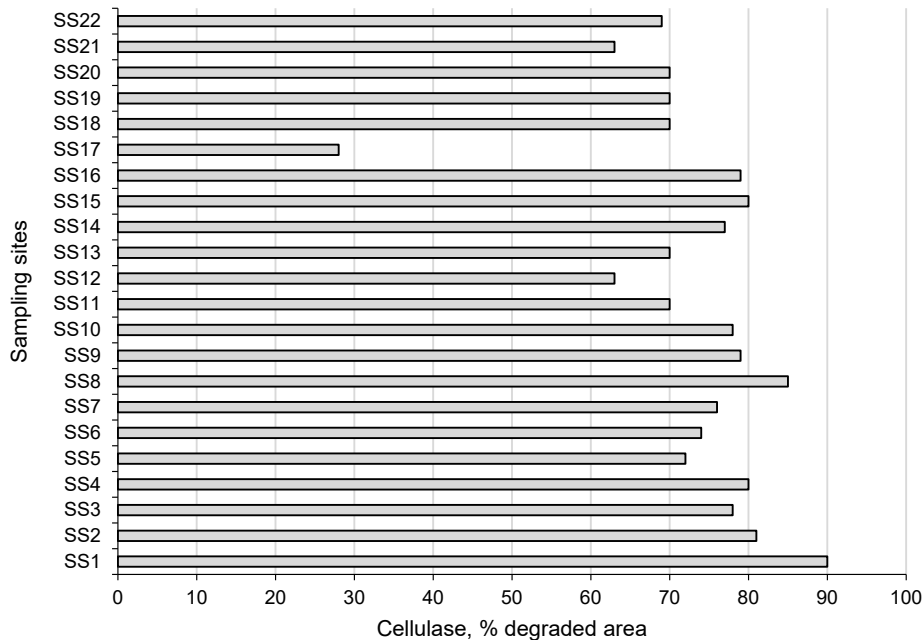


Fig. 13. Cellulase activity of soil microorganisms in summer (10–25 cm).

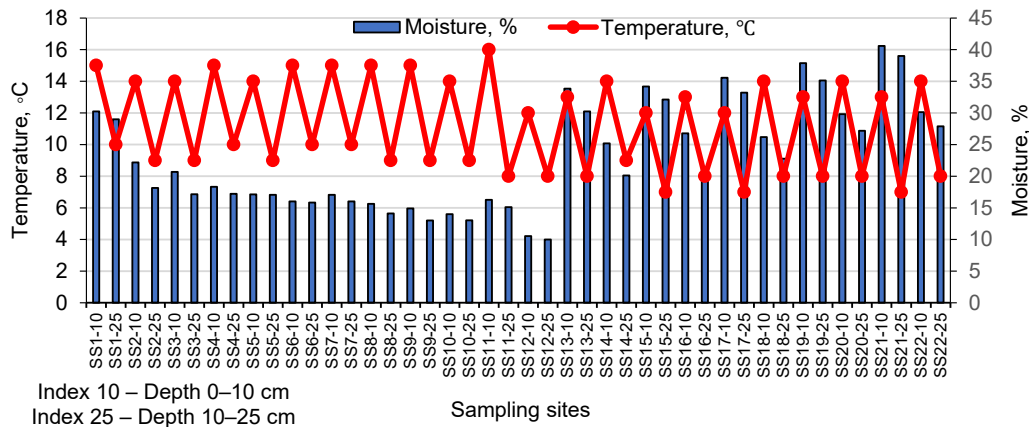


Fig. 14. Soil temperature and moisture in spring.

directly influenced by the mineral spring in Zheleznitza and lower values in the arable soils and the remaining sites of Zheleznitza and Dolni Pasarel.

In the sites of Vitosha Nature Park, the villages of Zheleznitza and Dolni Pasarel,

the soils have an acidic reaction, while the agricultural soils of Vrazhdebna district have a neutral reaction, which suggests an accumulation of biomass mainly of bacterial origin in the agricultural soils, and in those with an acidic reaction an in-

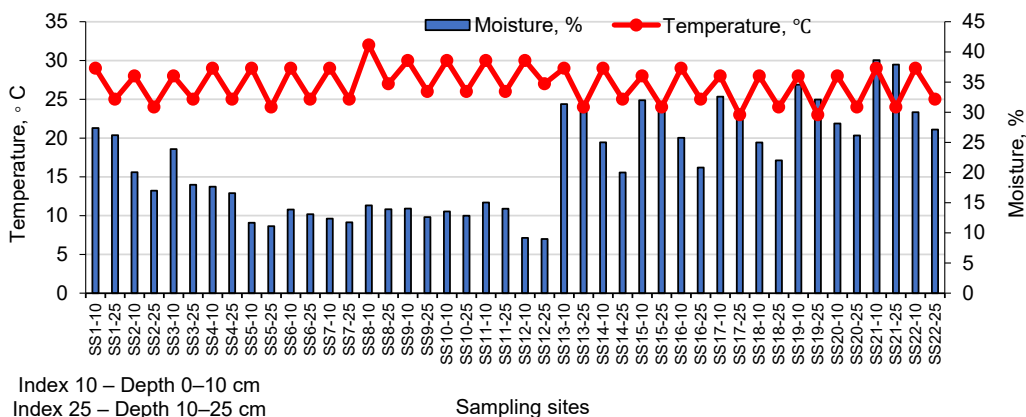


Fig. 15. Soil temperature and moisture in summer.

crease in the amount and activity of mould fungi, but on the other hand, anaerobic conditions in overmoistened soils limit the development of mycelial groups of micro-organisms (Fig. 16).

Microbial biomass carbon and enzyme activities depend moderately, positively, on soil temperature. Catalase activity depends significantly, and cellulase activity highly, positively, on the accumulated microbial biomass. A negative correlation is established between moisture and microbial biomass, as well as between moisture and enzyme activities, respectively significant negative between moisture and cellulase, moderate negative between

moisture and catalase and high negative between moisture and microbial biomass. A significant positive correlation is established between pH and catalase, and weak between pH and microbial biomass, while between cellulase and pH the correlation is moderate, negative (Table 2).

Coefficients with $p < 0.05$ are considered reliable predictors; the sign indicates direction (Table 3).

Welch's t-test showed significant seasonal differences in temperature ($p < 0.001$), while cellulase activity did not differ significantly between spring and summer ($p > 0.05$). Soil moisture was also higher in spring, but the difference was

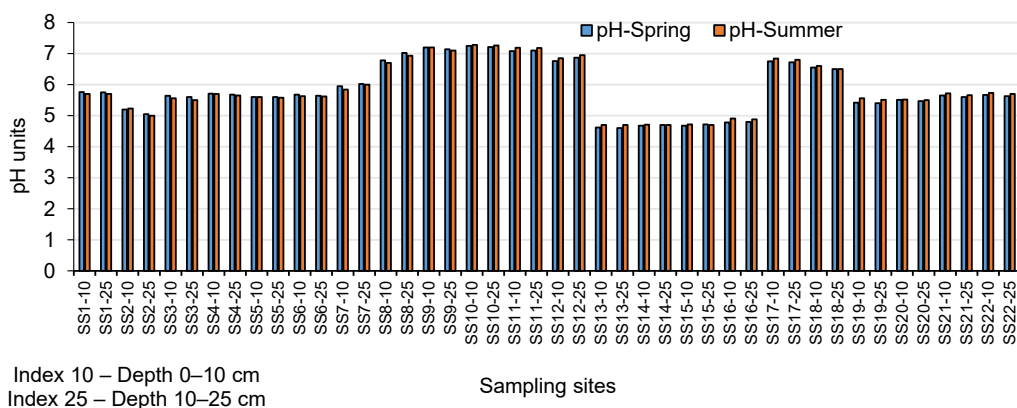


Fig. 16. Soil pH values.

Table 2. Correlation coefficients between biological and physicochemical soil parameters in spring and summer.

Variable	Depth	MBC	Catalase	Cellulase	Moisture	Temperature	pH
Spring							
Depth	1						
Microbial biomass C	-0.167	1					
Catalase	-0.110	0.5960	1				
Cellulase	-0.132	0.6563	0.4864	1			
Moisture	-0.130	-0.7296	-0.5277	-0.3279	1		
Temperature	-0.935	0.3786	0.2603	0.3127	-0.0724	1	
pH	-0.003	0.1483	0.5157	-0.2076	-0.4914	0.0728	1
Summer							
Depth	1						
Microbial biomass C	-0.136	1					
Catalase	-0.083	0.5074	1				
Cellulase	-0.137	0.6055	0.4355	1			
Moisture	-0.120	-0.6861	-0.4474	-0.2720	1		
Temperature	-0.900	0.2962	0.3374	0.2823	-0.1652	1	
pH	-0.010	0.0797	0.4905	-0.2561	-0.4508	0.2362	1

Table 3. *P*-values.

Predictor	Catalase (Spring)	Cellulase (Spring)	Catalase (Summer)	Cellulase (Summer)
Depth	0.649	0.069	0.188	0.024
MBC	0.065	0.163	0.055	0.026
pH	<0.001	<0.001	<0.001	<0.001
Temperature	0.547	0.052	0.227	0.010
Moisture	0.271	0.999	0.111	0.514
Cellulase	0.002		0.022	
Catalase		0.002		0.022

not statistically significant ($p > 0.05$). Catalase, microbial biomass carbon, and pH showed no significant seasonal variation (Table 4).

Multicollinearity diagnostics indicated acceptable levels for all predictors, with VIF values below 5 in most cases. Higher Variance Inflation Factor (VIF) values (>10) were observed for depth and temperature, reflecting their natural correlation structure, but tolerance values remained above 0.05. Slightly elevated collinearity

between temperature and moisture was noted, yet not at a level compromising model stability (Table 5)

Correlation analysis offers an initial indication of the direction and strength of relationships. However, in some cases variables that show significant correlations with enzyme activity may drop out of the regression model. The reasons include: (1) multicollinearity (strong association with another predictor), (2) lack of independent contribution once other factors are

Table 4. T-test results (spring vs summer).

Variable	Mean spring	Mean summer	t (Welch)	df (approx)	Sig. (2-tailed)
Temperature	11.23	26.91	-27.86	82.47	<0.001
Cellulase	78.55	74.43	1.45	82.68	0.152
Moisture	23.13	21.56	0.87	85.99	0.387
Catalase	3.16	3.10	0.69	85.95	0.490
MBC	365.36	357.70	0.48	85.98	0.631
pH	5.86	5.88	-0.11	86.00	0.912
Depth	17.50	17.50	0.00	86.00	1.000

Table 5. Multicollinearity diagnostics (VIF and tolerance).

Predictor	Catalase (Spring)	Cellulase (Spring)	Catalase (Summer)	Cellulase (Summer)
Depth	13.76 (Tol=0.073)	12.65 (Tol=0.079)	12.34 (Tol=0.081)	11.25 (Tol=0.089)
MBC	4.49 (Tol=0.222)	4.68 (Tol=0.214)	3.43 (Tol=0.291)	3.32 (Tol=0.301)
pH	1.81 (Tol=0.553)	2.28 (Tol=0.439)	2.22 (Tol=0.451)	2.09 (Tol=0.479)
Temperature	15.16 (Tol=0.066)	13.80 (Tol=0.072)	13.59 (Tol=0.074)	11.80 (Tol=0.085)
Moisture	3.96 (Tol=0.253)	4.09 (Tol=0.244)	3.63 (Tol=0.275)	3.85 (Tol=0.260)
Cellulase	2.34 (Tol=0.427)	—	2.57 (Tol=0.389)	—
Catalase	—	2.29 (Tol=0.437)	—	2.31 (Tol=0.434)

considered, or (3) the automatic stepwise selection procedure, which removes variables with $p > 0.05$. Soil moisture showed a negative and statistically significant correlation with catalase. Nevertheless, in the regression model this factor was excluded due to multicollinearity with temperature and pH. This indicates that the independent contribution of moisture is limited once other predictors are taken into account. In the correlation analysis, the relationship between pH and catalase is weak and non-significant. However, in the multiple regression model pH emerges as a significant positive predictor. This represents a classic example of a 'hidden effect': once the influences of temperature and mois-

ture are controlled for, the independent contribution of pH becomes statistically significant. Thus, pH is a key factor that is not revealed in a bivariate analysis but is crucial in a multivariate context. Stepwise multiple linear regression was applied to evaluate the factors influencing enzyme activities. This method is suitable when several potential predictors are available and their relative importance is not known in advance. The correlation analysis did not reveal extremely high intercorrelations ($r > 0.7$), but a moderate positive association was observed between microbial biomass and cellulase, with $r \approx 0.66$ in spring and $r \approx 0.61$ in summer. This indicates a certain degree of interdependence among

some variables. The stepwise procedure sequentially adds or removes predictors based on their statistical significance and independent contribution, thereby avoiding model oversaturation and reducing the potential impact of multicollinearity. Thus, an optimal balance is achieved between explanatory power and model simplicity (Table A1 in Appendix A).

The regression model for catalase in spring is given by equation (1):

$$\begin{aligned} \text{Catalase} = & -0.5359 - 0.0091 \cdot \text{Depth} + \\ & + 0.0022 \cdot \text{MBC} + 0.3497 \cdot \text{pH} - \\ & - 0.0331 \cdot \text{Temperature} + 0.0106 \cdot \text{Moisture} + \\ & + 0.0145 \cdot \text{Cellulase}, \end{aligned} \quad (1)$$

where: pH was identified as a significant positive predictor ($B \approx 0.35$, $p < 0.001$), while cellulase activity also contributed significantly and positively. Microbial biomass carbon (MBC) had a positive but marginal effect ($p \approx 0.065$), and depth, temperature, and moisture were not significant. Although MBC correlated positively with catalase in the correlation analysis, this relationship lost significance in the regression model, likely due to overlapping influence among predictors (multicollinearity).

The corresponding regression for cellulase activity in spring is presented in equation (2):

$$\begin{aligned} \text{Cellulase} = & 1.5432 + 1.2005 \cdot \text{Depth} + \\ & + 0.0562 \cdot \text{MBC} - 9.6367 \cdot \text{pH} + \\ & + 3.5300 \cdot \text{Temperature} - 0.0005 \cdot \text{Moisture} + \\ & + 16.5418 \cdot \text{Catalase}, \end{aligned} \quad (2)$$

where: depth, temperature, and catalase activity emerged as strong positive predictors, whereas pH had a strong negative effect ($B \approx -9.64$, $p < 0.001$). MBC and soil moisture were not significant contributors. Despite the moderate positive correlation between MBC and cellulase ($r \approx 0.66$), this effect disappeared in the multivariate model due to stronger contributions from

other predictors, reflecting a shared or mediated relationship rather than a direct one.

During summer, the model for catalase activity took the form shown in equation (3):

$$\begin{aligned} \text{Catalase} = & -3.3941 + 0.0262 \cdot \text{Depth} + \\ & + 0.0021 \cdot \text{MBC} + 0.2998 \cdot \text{pH} + \\ & + 0.0812 \cdot \text{Temperature} + 0.0156 \cdot \text{Moisture} + \\ & + 0.0135 \cdot \text{Cellulase}, \end{aligned} \quad (3)$$

where: pH ($B \approx 0.30$, $p < 0.001$) and cellulase activity were significant positive predictors. Depth, MBC, temperature, and soil moisture showed positive but statistically non-significant effects. The positive yet non-significant temperature coefficient ($B \approx 0.0812$, $p > 0.05$) indicates that the observed correlation between temperature and catalase is explained largely by covariance with other predictors – particularly pH and cellulase – rather than by an independent effect.

Finally, cellulase activity in summer was described by equation (4):

$$\begin{aligned} \text{Cellulase} = & -74.4463 + 1.1870 \cdot \text{Depth} + \\ & + 0.0650 \cdot \text{MBC} - 8.6619 \cdot \text{pH} + \\ & + 4.5112 \cdot \text{Temperature} + 0.1750 \cdot \text{Moisture} + \\ & + 9.8751 \cdot \text{Catalase}, \end{aligned} \quad (4)$$

where: temperature, MBC, and catalase activity acted as significant positive predictors, while pH remained a significant negative predictor, consistent with the spring model. Depth and soil moisture were not significant. Notably, depth contributed significantly in spring but not in summer, suggesting a seasonal pattern in which the influence of depth diminishes under warmer and drier summer conditions. The non-significance of soil moisture may again be attributed to its strong correlation with temperature.

Across the four models, pH consistently emerges as a key factor – positive for catalase and negative for cellulase. Al-

though the simple correlation between pH and catalase is weak and non-significant, in regression analysis pH becomes a significant predictor, representing a classic example of a 'hidden effect'. Temperature strongly stimulates cellulase and shows a stable positive role, while for catalase its influence disappears once other factors are considered. Microbial biomass carbon supports both enzymes, particularly in summer, with a more pronounced contribution to cellulase activity. The inclusion of one enzyme as predictor for the other highlights the interdependence of enzymatic activities and the complex nature of soil biochemical processes. Overall, step-wise multiple regression enabled the identification of the most relevant independent factors, while avoiding model oversaturation and reducing the risk of multicollinearity, thus providing a more reliable and transparent interpretation of the role of individual predictors.

The relationships established in the present study confirm that enzyme activities are closely linked to the microbiological and chemical properties of the soil, which corresponds to the results of Wang et al. (2023), who reported that enzyme activities are mainly determined by soil nutrient status and the content of microbial biomass carbon and nitrogen, with biomass nitrogen being particularly important for cellulase. In both studied seasons, pH emerged as a key predictor of catalase and cellulase activity, although with opposite directions of influence. Although higher temperatures generally enhance enzymatic reactions, pH may restrict catalytic efficiency (Souza and Billings 2021), while in our study it limited cellulase activity. Under more stressful or waterlogged conditions, the reduced effect of pH in our data resembles the trends described by Chander and Joergensen (2001), who

noted that pH often loses significance when stronger stressors such as toxic metals or oxygen deficiency prevail. The inclusion of depth, pH, temperature and microbial biomass among the consistent predictors in our models corresponds to the synthesis by Daunoras et al. (2024), who emphasised that these parameters jointly determine microbial community activity and enzymatic potential.

Conclusions

The obtained results showed that microbial and enzymatic activities respond sensitively to land use, soil depth, and environmental conditions. In spring, microbial biomass carbon (MBC) reached approximately $360 \text{ mg C} \cdot \text{kg}^{-1}$ and was about 2–3 times higher in the agricultural soils of Vrazhdebna and in the soil near the mineral spring at Zheleznitsa compared with the forest soils of Vitosha, reflecting the effects of cultivation and irrigation. The activities of catalase and cellulase were also highest in spring and greater in agricultural soils, decreasing with depth and under waterlogged conditions. Catalase activity reached up to $3.7 \text{ L O}_2 \cdot \text{g}^{-1} \cdot 30 \text{ min}^{-1}$, while complete cellulose degradation (100%) was recorded in the most biologically active soils.

Soil moisture exerted a strong negative effect on MBC ($r \approx -0.73$) and enzyme activities, whereas temperature and pH acted in opposite directions: temperature promoted ($r \approx 0.28$ – 0.38), while pH limited cellulase activity ($r \approx -0.21$ to -0.26) but enhanced catalase ($r \approx 0.49$ – 0.52). Regression analysis confirmed the role of pH as a stable predictor—positive for catalase ($B \approx +0.30$ – 0.35 ; $p < 0.001$) and negative for cellulase ($B \approx -8.7$; $p < 0.001$) in both seasons. In the spring

model for catalase, pH and cellulase activity emerged as significant positive predictors, whereas in summer pH and cellulase again remained significant, while depth, temperature, moisture, and MBC were positive but non-significant. For cellulase in spring, catalase activity was the strongest positive predictor ($B \approx +16.5$; $p = 0.002$), pH had a strong negative effect ($B \approx -9.6$; $p < 0.001$), and temperature and depth had positive effects at the margin of significance. In summer, cellulase was significantly stimulated by temperature ($B \approx +4.5$; $p = 0.010$), MBC ($B \approx +0.065$; $p = 0.026$), depth ($B \approx +1.19$; $p = 0.024$), and catalase ($B \approx +9.88$; $p = 0.022$), with pH remaining a negative predictor.

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Appendix A

Table A1. Regression analysis.

Model summary – Catalase (spring)									
R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson	N				
0.817	0.668	0.614	0.266	0.797	44				
ANOVA									
Source	Sum of Squares	df	Mean Square	F	Sig.				
Regression	5.2650	6	0.8775	12.40	<i>p</i> <0.001				
Residual	2.6179	37	0.0708						
Total	7.8829	43							
Coefficients									
Term	B	Std. Error	Standardised Beta	t	Sig.	95% CI Lower	95% CI Upper	Tolerance	VIF
Intercept	-0.5359	1.1164		-0.48	0.634	-2.7979	1.7261		
Q(Depth)	-0.0091	0.0198	-0.162	-0.46	0.649	-0.0493	0.0311	0.073	13.76
Q(MBC)	0.0022	0.0011	0.381	1.90	0.065	-0.0001	0.0045	0.222	4.494
Q(pH)	0.3497	0.0654	0.681	5.35	<0.001	0.2172	0.4822	0.553	1.808
Q(Temperature)	-0.0331	0.0544	-0.224	-0.61	0.547	-0.1435	0.0772	0.066	15.16
Q(Moisture)	0.0106	0.0095	0.210	1.12	0.271	-0.0086	0.0298	0.253	3.957
Q(Cellulase)	0.0145	0.0042	0.495	3.42	0.002	0.0059	0.0231	0.427	2.342
Model summary – Cellulase (spring)									
R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson	N				
0.822	0.675	0.623	8.984	1.170	44				
ANOVA									
Source	Sum of Squares	df	Mean Square	F	Sig.				
Regression	6216.5	6	1036.0905	12.84	<i>p</i> <0.001				
Residual	2986.4	37	80.7126						
Total	9202.9	43							
Coefficients									
Term	B	Std. Error	Standardised Beta	t	Sig.	95% CI Lower	95% CI Upper	Tolerance	VIF
Intercept	1.5432	37.8229		0.04	0.968	-75.09	78.18		
Q(Depth)	1.2005	0.6421	0.623	1.87	0.069	-0.101	2.5016	0.079	12.65
Q(MBC)	0.0562	0.0395	0.288	1.42	0.163	-0.024	0.1363	0.214	4.676
Q(pH)	-9.6367	2.4779	-0.549	-3.89	<0.001	-14.66	-4.6160	0.439	2.276
Q(Temperature)	3.5300	1.7547	0.700	2.01	0.052	-0.025	7.0854	0.072	13.80
Q(Moisture)	-0.0005	0.3251	-0.000	-0.00	0.999	-0.659	0.6583	0.244	4.091
Q(Catalase)	16.542	4.8411	0.484	3.42	0.002	6.7329	26.351	0.437	2.289

Model summary – Catalase (summer)

R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson	N
0.790	0.624	0.563	0.276	0.797	44

ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Regression	4.6757	6	0.7793	10.23	$p<0.001$
Residual	2.8190	37	0.0762		
Total	7.4948	43			

Coefficients

Term	B	Std. Error	Standardised Beta	t	Sig.	95% CI Lower	95% CI Upper	Tolerance	VIF
Intercept	-3.3941	2.0065		-1.69	0.099	-7.4596	0.6715		
Q(Depth)	0.0262	0.0195	0.475	1.34	0.188	-0.0133	0.0656	0.081	12.34
Q(MBC)	0.0021	0.0011	0.371	1.98	0.055	-0.0000	0.0042	0.291	3.433
Q(pH)	0.2998	0.0753	0.598	3.98	<0.001	0.1472	0.4524	0.451	2.220
Q(Temperature)	0.0812	0.0660	0.457	1.23	0.227	-0.0526	0.2149	0.074	13.59
Q(Moisture)	0.0156	0.0095	0.314	1.63	0.111	-0.0038	0.0349	0.275	3.631
Q(Cellulase)	0.0135	0.0057	0.385	2.38	0.022	0.0020	0.0249	0.389	2.572

Model summary - Cellulase (summer)

R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson	N
0.814	0.663	0.608	7.472	0.943	44

ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Regression	4063.0	6	677.1697	12.13	$p<0.001$
Residual	2065.8	37	55.8318		
Total	6128.8	43			

Coefficients

Term	B	Std. Error	Standardised Beta	t	Sig.	95% CI Lower	95% CI Upper	Tolerance	VIF
Intercept	-74.446	55.0326		-1.35	0.184	-185.95	37.060		
Q(Depth)	1.1870	0.5039	0.754	2.36	0.024	0.1661	2.2079	0.089	11.254
Q(MBC)	0.0650	0.0281	0.403	2.32	0.026	0.0081	0.1219	0.301	3.318
Q(pH)	-8.6619	1.9774	-0.604	-4.38	<0.001	-12.668	-4.6554	0.479	2.087
Q(Temperature)	4.5112	1.6650	0.888	2.71	0.010	1.1375	7.8849	0.085	11.800
Q(Moisture)	0.1750	0.2656	0.123	0.66	0.514	-0.3633	0.7132	0.260	3.848
Q(Catalase)	9.8751	4.1436	0.345	2.38	0.022	1.4793	18.271	0.434	2.305

Microbial activity as an indicator of ecosystem functioning: a case study from two spruce forest soils

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Thematic field: Ecosystem biodiversity, conservation and environmental restoration

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Abstract

Forests are highly productive ecosystems that act as carbon sinks, where soil organic matter is formed from the residues of biomass decomposition as well as carbon deposited by the rhizosphere. Soil microorganisms play a crucial role in these processes by decomposing plant litter, thereby causing changes in the physical and chemical properties of the soil. This study aimed to determine whether soil bacterial activity can serve as an indicator of soil health and forest condition. In this study, Biolog EcoPlate™ assay was used to analyse the community-level physiological profiles (CLPPs) from spruce forest soils. Samples were taken from two plots in coniferous forests with soils classified as Dystric *Cambisols* (Sample plot 1, SP1) and Eutric *Cambisols* (Sample plot 2, SP2) in three depths (0–10, 10–20 and 20–30 cm). Soils were sandy clay loam and clay loam, respectively. Average microbial activity (expressed as the area under the curve, AUC) was nearly twice as high in SP2 as in SP1. In SP1 highest activity was measured at the 10–20 cm depth, while in SP2 it was measured at 20–30 cm. CLPP showed higher bacterial affinity for polymers and lower for carbohydrates. There was a significant difference in the metabolic activity among carbon guilds utilisation ($p < 0.05$) but not among different depths ($p > 0.1$). Tween 40, Tween 80 and γ -aminobutyric acid were preferentially utilised, while 2-hydroxybenzoic acid, α -ketobutyric acid and glucose-1-phosphate had the lowest utilisation rates. The strong linear correlation between microbial Shannon diversity and Evenness indices showed that depth influenced these rates, but not the number of utilised carbon sources. Results indicate that microbial activity can serve as a quick and reliable indicator of forest ecosystem functioning.

Key words: bacterial functional diversity, bacterial metabolic activity, Biolog EcoPlate™, community-level physiological profiles, soil bacteria.

Introduction

Forests are natural ecosystems that produce a variety of ecosystem services such as biomass production, habitat provisioning, pollination, seed dispersal, wind resistance, fire and pest regulation, carbon sequestration, and cultural ecosystem services (Brockerhoff et al. 2017). This requires their preservation (Pawlik et al. 2020) in order to continue providing these services. Soil microorganisms play a crucial role in forest ecosystems since they participate in key processes such as nutrient cycling (Paul 2007), organic matter transformation (Allison et al. 2013), and improving soil structure and fertility (Schneider et al. 2012), and thus influencing plant growth, and ecosystem succession (Zhang et al. 2012). On the other hand, plants provide litter and root exudates that serve as carbon sources for microorganisms (Kiikkilä et al. 2006), and can also affect soil physicochemical properties (Menyailo et al. 2002). Soil functional diversity depends on a variety of factors such as vegetation type (Gałazka et al. 2022), quality and quantity of leaf (Myers et al. 2001) and root (Berg and McClaugherty 2014, Herzog et al. 2019) litter, organic carbon content (Bending et al. 2002), soil structure and physicochemical properties (Hu et al. 2010), climate (Paul 2007) and different land management practices (Li et al. 2024) which makes it indicative for forest health. Functional features of microbial communities can be helpful in understanding the environment quality and forest stand condition (Drenovsky et al. 2004, Grayston and Prescott 2005, Fierer and Jackson 2006) due to the microorganisms' ability to respond quickly to environmental changes.

Thus, analysing soil microbial activity is crucial for the functioning of forest ecosystems and for applying proper management practices.

One of the contemporary methods used for assessing bacterial functional diversity is the community level physiological profiling (CLPP) technique (Garland and Millis 1991). In particular, the Biolog EcoPlate™ system is a simple and relatively cost-effective tool for such analyses, which contains 31 naturally occurring carbon substrates in triplicate (Insam 1997). It enables to evaluate the ability of microorganisms to utilise different carbon substrates, and thus determining the 'metabolic footprint' of the microbial communities. Since this method is very sensitive in determining soil functional dynamics, it has been applied by many authors to soils with different vegetation types (Gałazka et al. 2022), management practices (Li et al. 2024), altitude (Han et al. 2015), ages (Lan et al. 2019) and disturbances (Barros et al. 2023).

The purpose of the present study was to identify relationships between soil bacterial activity, particularly the utilisation of specific carbon sources and forest condition. We aimed to investigate microbial activity at different soil depths in two spruce-dominated stands as an early functional indicator of undesirable changes such as decline and/or decay in undisturbed forests. This provided greater insights into the functional relationships between microorganisms and trees in forest ecosystems. We hypothesised that microbial activity could serve as an early indicator of forest health and ecosystem functioning, and could be incorporated as an additional functional parameter in forest monitoring programmes.

Material and Methods

Sampling site and procedure

Soil sampling was conducted in April 2024 from two plots using a digging shovel. The sample plots are located in even-aged, monodominant Norway spruce (*Picea abies* (L.) Karst) forests in Southern Bulgaria. Sample plot 1 (SP1, region of Smolyan) is situated at 41°37'10.01" N, 24°39'25.64" E, at an elevation of 1665 m a.s.l. and represents a 90-year-old stand. Sample plot 2 (SP2, region of Pamporovo) is located at 41°39'54.00" N, 24°42'57.00" E, 1420 m a.s.l. with an 80-year-old stand (Fig. 1).

At each plot, soil samples were composited from 5 subsites within a 10 × 10 m square plot. Each composite sample was collected from the respective depth

(0–10 cm: A soil horizon; 10–20 cm and 20–30 cm: B horizon) into sterile container and transported at 4 °C to laboratory for further analyses.

Soil physicochemical parameters

Soil parameters were determined as follows: soil texture was measured as clay – <2 µm; silt – 2–63 µm and sand – 63–2,000 µm (ISO 11277:2009 2009), soil pH_{H2O} was measured according to ISO 10390:2005 (2005). The content of soil organic matter was determined by a modified Turin method – 120 °C, 45 min and CuSO₄ as a catalyst (Kononova 1966, Filcheva and Tsadilas 2002). Total nitrogen content was measured using the Kjeldahl method with Kjeltac Auto 1030 analyser. Soil temperature at each depth was measured *in situ* with a digital ther-



Fig. 1. Site location map of the SP1 (region of Smolyan) and SP2 (region of Pamporovo) sampling plots in Southern Bulgaria.

mometer (EC-88010).

Soil microbial activity

Soil bacterial activity was analysed using Biolog EcoPlate™ (Biolog, Inc., Hayward CA, USA) assay. The microplates contain 31 carbon sources (CSs) classified into five carbon guilds (CGs) (Weber and Legge 2009): carbohydrates (CH), carboxylic acids (CA), polymers (Polym), amino acids (AA), and amines (Amin) each in triplicate and 3 wells for control. Soil suspensions were prepared by shaking the samples in sterile 0.9 % NaCl solution at a ratio 1:10 for 1 h at 160 rpm. Suspensions were filtered through an 8 µm polycarbonate filter (Whatman Nucleopore, ø 47 mm) to remove larger soil particles. The microplates were inoculated with 120 µL of the filtrate and incubated at 22 ± 1 °C in the dark. Optical densities (ODs) of the wells were measured every 24 h for 5 days at 590 nm on a Microplate Reader (LKB 5060-006) with software package DV990 'Win 6'. The area under the curve (AUC), in square units (SU), was calculated from the obtained kinetic data and applied to evaluate the average well colour development (AWCDs), community-level physiological profiles (CLPPs), and functional diversity (H') and evenness (E) indices.

Area under curve (AUC) was calculated according to equation (1):

$$AUC = \frac{\sum OD_n + OD_{n+1}}{2 \cdot (t_{n+1} - t_n)}, \quad (1)$$

where: OD_n and OD_{n+1} represent the optical densities at two consecutive measurements at times t_n and t_{n+1} (Guckert et al. 1996).

Equation (2) was used for calculating the average well colour development (AWCD):

$$AWCD = \frac{\sum_{i=1}^N AUC_i}{N}, \quad (2)$$

where: AUC_i is the area under the curve of the i -th CS, and N is the number of CSs in the microplate ($N = 31$) (Garland and Mills 1991).

Shannon-Weaver (H') and evenness (E) indices were calculated according to equations (3) and (4):

$$H = \sum_{i=1}^S p_i \cdot \ln(p_i), \quad (3)$$

$$E = \frac{e^H}{S}, \quad (4)$$

where: p_i is the ratio of the AUC of the i -th CS to the total AUC of all CSs on the microplate, and S is the number of carbon sources with non-zero utilisation (Magurran 1988).

The control AUC value was subtracted from the AUC of each CS prior to the calculation.

Statistical analysis

One-way analysis of variance (ANOVA) was applied to examine differences among the measured parameters. Correlation analysis was performed using the PAST software package (Hammer et al. 2001). All statistical analyses were conducted at a significance level of $p < 0.05$.

Results and Discussion

Soils were classified as Dystric *Cambisols* (SP1) and Eutric *Cambisols* (SP2) according to IUSS Working Group WRB (2022). Soils in SP1 had a sandy clay loam texture (sand: 62.81–66.53 %, silt: 15.67–16.96 %, clay: 17.80–20.60 %), and SP2 had a clay loam texture (sand:

26.91–32.09 %, silt: 29.53–31.98 %, clay: 37.77–41.11 %). Average soil pH for SP1 was 4.6 ± 0.1 , while for SP2 was higher 5.8 ± 0.6 (Table 1).

The difference between pH for the two sample plots was statistically significant ($p < 0.01$), and also among the depths in SP2 ($p < 0.01$), but no significant difference was found among the depths for SP1 ($p > 0.13$). Galvan et al. (2008) reported pH values in Norway spruce ecosystems in the Alps from 4.2 to 6.3. Samec et al. (2023) reported values of 4.25 ± 1.12 for spruce (*Picea abies* Karst.) in Central Europe. Fabiánek et al. (2009) reported lower pH values for soils under spruce stand in the Czech Republic which may indicate anthropogenic acidification of the soil profile. Soil temperature varied in a narrow range between sample plots and depths with average values from 5.38 ± 0.46 °C for SP1 to 5.64 ± 0.57 °C for SP2. For both sample plots, it decreased with increasing depth ($r = -0.98$, $p < 0.01$ for SP1; $r = -0.96$, $p < 0.01$ for SP2), and the differences among depths were statistically significant ($p < 0.01$). However, no significant differences in soil temperature were observed between the two sample plots ($p > 0.24$). These results are in accordance with the tendency for decrease observed from Kohútová and Štykar (2024) for soil temperature in April. The same trend was observed for the humus content in depth as there were statistically significant neg-

ative correlations for the two sample plots ($r = -0.93$, $p < 0.01$ for SP1; $r = -0.95$, $p < 0.01$ for SP2). Here, again the differences among depths were statistically significant ($p < 0.01$), while no significant difference in the humus content between the two sample plots ($p > 0.64$) was found. These results for humus content are lower than those reported from Menšík et al. (2015) in the range from 12.24 to 12.76 % for H horizon and from 11.64 to 11.90 % for Ah horizon for spruce forests in the Highlands area of the Czech Republic and to those reported by Grigorov and Assenov (2018) for Eutric *Cambisols* in Mala Planina, Bulgaria. Measured humus concentrations are in accordance with the results reported by Kroyan (2018) for *Cambisols* in Armenia which varied from 2.8 to 11.2 %. The humus concentration is slightly higher than the values reported by Dospatliev et al. (2018) in *Cambisols* soils from the Batak Mountain, Bulgaria and by Bensa et al. (2013) Eutric *Cambisols* (2.23 %) in continental Croatia. The variations in humus content may be a result of the complex interactions between vegetation composition (Zanella et al. 2018), soil characteristics (Ponge et al. 2011), soil micro- and macroorganisms' species and activities (Xu et al. 2015) and environmental factors (Zanella et al. 2018). The organic carbon ($r = -0.93$ for SP1 and $r = -0.95$ for SP2) and total nitrogen ($r = -0.98$ for SP1 and SP2) concentrations

Table 1. Physicochemical parameters of the sample plots in depth.

Sample plot depth, cm	pH H ₂ O	Temperature, °C	Humus, %	Organic carbon, %	Total nitrogen, %	C/N ratio
SP1 (0–10 cm)	4.6	5.88 ± 0.29	6.50	3.77	0.274	14
SP1 (10–20 cm)	4.7	5.28 ± 0.22	4.94	2.86	0.174	16
SP1 (20–30 cm)	4.5	4.98 ± 0.24	4.62	2.68	0.127	21
SP2 (0–10 cm)	5.3	6.28 ± 0.54	8.28	4.80	0.455	11
SP2 (10–20 cm)	5.7	5.46 ± 0.19	3.87	2.25	0.275	8
SP2 (20–30 cm)	6.5	5.18 ± 0.08	2.61	1.51	0.159	10

decreased with increasing depth as there was a significant difference in the values among the depths ($p < 0.01$). A statistically significant difference was observed for the total nitrogen and C/N ratio between the sample plots, but not for the organic carbon content ($p = 0.64$).

The average well colour development, expressed as the area under the curve, represents the overall capacity of the microbial community to utilise carbon sources (Degens et al. 2000). The average metabolic activity of SP2 was 86 % higher than that of SP1. The highest activity for SP1 was measured in the 10–20 cm depth (mean: 0.81 ± 0.03), and for SP2 it was registered in the 0–10 cm and 20–30 cm depths (mean: 1.28 ± 0.36 and 1.34 ± 0.13 , respectively). There was a statistically significant difference among the activity in depths for both sample plots ($p < 0.05$).

Although both sample plots are situated in spruce forests, the differences in bacterial activity may be related to various factors such as carbon-to-nitrogen ratio (Springob and Kirchmann 2003), or secretion of metabolic intermediates and secondary metabolites, which increases with forest age and may suppress soil microorganisms (Lan et al. 2019). Pinho et al. (2020) also reported changes in the soil microbiome as a result of alterations in soil pH due to forest disease. Furthermore, Lan et al. (2019) concluded that forest age influences the soil microbial community structure and functions in eucalyptus plantations. This supports the relationship among tree health, the soil microbiome, and the belowground environment (Mercado-Blanco et al. 2018), and can be used to develop appropriate strategies for forest management.

The utilisation rates of the carbon guilds followed a similar trend in both

sample plots but were higher in SP2 compared to SP1 (Fig. 2). The relative increases in SP2 were as follows: polymers – 24.3 %, carbohydrates – 74.7 %, carboxylic acids – 110 %, amino acids – 117.3 %, and amines – 197.46 %. In SP1, the highest share of metabolic activity was observed for polymers (38 %), while amines and carbohydrates had the lowest shares (12 % and 13 %, respectively). In SP2, polymers and amino acids accounted for the highest proportions (25 % each), whereas carbohydrates had the lowest (12 %). The utilisation rates of the carbon guilds differed statistically both within and between the sample plots ($p < 0.01$).

The utilisation rates of all carbon guilds in SP1 were highest at the 10–20 cm depth (Fig. 2A). The lowest AWCD values were registered for carbohydrates, polymers and amino acids at 0–10 cm depth, while for carboxylic acids and amines the minimum occurred at 20–30 cm. The analysis revealed a negative correlation between utilisation rate and depth ($r = -0.08$), which was not statistically significant ($p = 0.84$). In SP2, the 10–20 cm layer showed the lowest utilisation rates for all carbon guilds except amines (Fig. 2B). The highest activity for carbohydrates and amino acids was observed at 0–10 cm, while polymers, carboxylic acids, and amines peaked at 20–30 cm. Statistical analysis indicated a positive, though non-significant, correlation between activity and depth ($r = 0.10$; $p = 0.80$).

The polymers and amino acids carbon guilds showed the highest AWCD values in both sample plots (Fig. 3). Despite this observation, the carbon guilds exhibited different trends in their utilisation rates with depth: 1) a significant increase in polymers utilisation in SP1; 2) a moderate increase in amino acid and carbohydrate

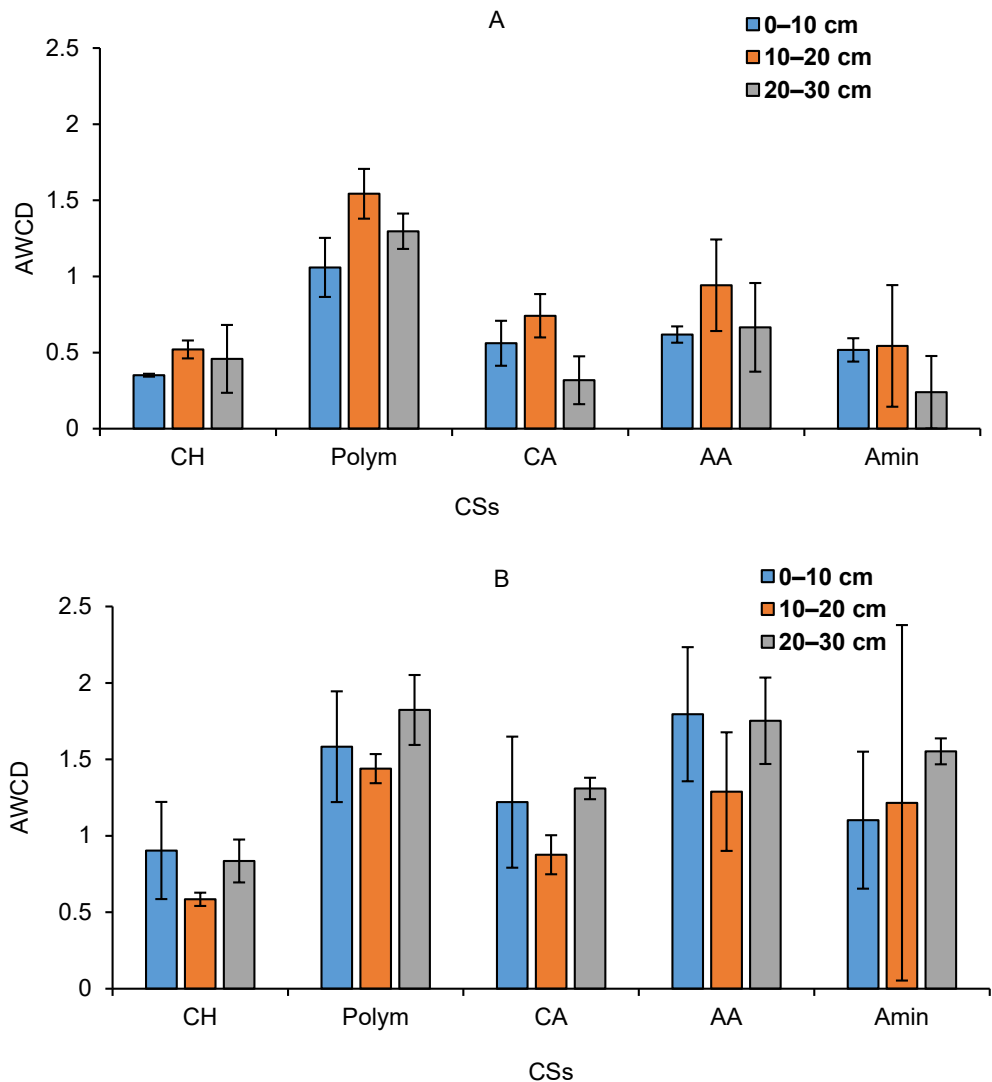


Fig. 2. Average utilisation rates of carbon guilds at different sampling depths in SP1 (A) and SP2 (B).

Note: CSs – carbon sources: CH – carbohydrates; Polym – polymers; CA – carboxylic acids; AA – amino acids; Amin – amines. AWCD – average well colour development.

utilisation in SP1, accompanied by a slight decrease in amine and carboxylic acid utilisation in SP1 and in amino acids in SP2; and 3) no clear trend in the utilisation rates of amines, carbohydrates, carboxylic ac-

ids, and polymers in SP2. A statistically significant difference was found among the different carbon guilds ($p < 0.01$), but not among their AWCD values across the three depths within each sample plot

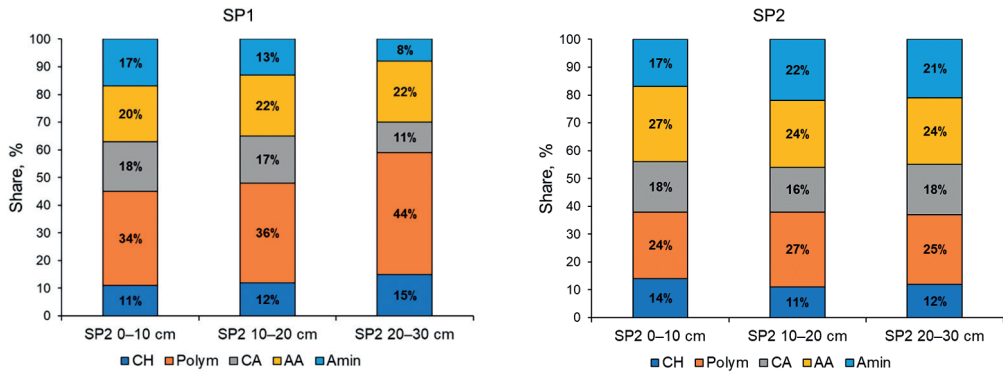


Fig. 3. Carbon guilds utilisation share at different depths in SP1 (left) and SP2 (right), expressed as a percentage of total metabolic activity.

Note: CH – carbohydrates; Polym – polymers; CA – carboxylic acids; AA – amino acids; Amin – amines.

($p > 0.14$ for SP1; $p > 0.12$ for SP2).

Soil microorganisms participate in key processes such as nutrient cycling, tree nutrition and plant health, due to the mobilization and transfer of nutrients via the food web (Uroz et al. 2016). The type of vegetation determines the microbial biodiversity and hence its metabolic activity (Si et al. 2018). Gałazka et al. (2022) observed higher utilisation of amines and amides, amino acids and polymers from bacteria in bulk soils from *Alnus glutinosa* (L.) Gaertn. stands, carboxylic and acetic acids in *Betula pendula* stands, and carbohydrates in *Pinus sylvestris* L. stands. Furthermore, Türiş and Candan (2022) reported that the microbial community in *Fagus-Abies* forest utilised carboxylic acids, polymers, amines, amino acids, and carbohydrates; the microbiota from *Quercus-Fagus* forest utilised carboxylic acids, polymers, amino acids, and carbohydrates. Higher utilisation of carbohydrates, but lower of amines/amides was registered in Luvisols from the forest territory of Western Stara Planina Mountain in Bulgaria (Grigorova-Pesheva 2023) and in

the rhizosphere of eucalyptus forest (Lan et al. 2019). The preferential utilisation of polymers and amino acids may be due to the fact that they are present in root exudates and provide substrates for soil microorganisms (Behera et al. 2018). Moreover, other authors have reported that soil microbial activity depends on plant species and soil properties (Wolińska et al. 2020), as bulk soil is characterised by active nutrient cycling and the formation of diverse microbial communities (Grządziel et al. 2019, Woźniak and Gałazka 2019).

Among the carbon sources C1 (Tween 40) and D1 (Tween 80) from the polymers and E3 (γ -aminobutyric acid) from the carboxylic acids were the most preferentially utilised with AWCD values of 2.28 ± 0.53 , 2.10 ± 0.57 and 2.11 ± 0.89 , respectively (Table 2). The carboxylic acids C3 (2-hydroxybenzoic acid) and G3 (α -ketobutyric acid) and the carbohydrate G2 (glucose-1-phosphate) were the least utilised sources with AWCD of 0.16 ± 0.18 , 0.05 ± 0.06 and 0.12 ± 0.16 , respectively.

Furthermore, D1, E3 and B4 sources exerted more than 100 % higher activity

Table 2. Community-level physiological profiles (CLPPs) of bacterial communities in SP1 and SP2 soils at different depths.

Carbon sources	SP1	SP1	SP1	SP2	SP2	SP2	CGs
	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20–30 cm	
G1 D-Cellobiose	Red	Red	Red	Orange	Red	Blue	CH
H1 α -D-Lactose	Red	Red	Red	Orange	Orange	Blue	
A2 β -Methyl-D-glucoside	Red	Red	Red	Red	Red	Blue	
B2 D-Xylose	Orange	Orange	Yellow	Orange	Yellow	Green	
C2 i-Erythritol	Red	Red	Red	Yellow	Red	Orange	
D2 D-Mannitol	Blue	Blue	Blue	Blue	Blue	Red	
E2 N-Acetyl-D-glucosamine	Blue	Blue	Blue	Blue	Blue	Orange	
G2 Glucose-1-phosphate	Red	Red	Red	Orange	Red	Red	
H2 D,L- α -Glycerol phosphate	Yellow	Orange	Orange	Orange	Red	Orange	Polym
C1 Tween 40	Blue	Blue	Blue	Blue	Blue	Orange	
D1 Tween 80	Blue	Blue	Blue	Blue	Blue	Blue	
E1 α -Cyclodextrin	Blue	Blue	Blue	Green	Green	Blue	
F1 Glycogen	Orange	Red	Orange	Orange	Orange	Green	CA
B1 Pyruvic acid methyl ester	Blue	Blue	Blue	Blue	Blue	Red	
F2 D-Glucosaminic acid	Yellow	Blue	Orange	Green	Blue	Red	
A3 D-Galactonic acid γ -lactone	Blue	Blue	Red	Blue	Blue	Blue	
B3 D-Galacturonic acid	Blue	Yellow	Orange	Blue	Blue	Blue	
C3 2-Hydroxybenzoic acid	Red	Red	Red	Orange	Red	Red	
D3 4-Hydroxybenzoic acid	Orange	Orange	Orange	Red	Red	Red	
E3 γ -Aminobutyric acid	Blue	Blue	Blue	Blue	Blue	Blue	AA
F3 Itaconic acid	Yellow	Red	Blue	Orange	Red	Red	
G3 α -Ketobutyric acid	Red	Red	Red	Red	Red	Red	
H3 D-Malic acid	Red	Red	Orange	Orange	Red	Orange	
A4 L-Arginine	Blue	Blue	Blue	Blue	Blue	Green	
B4 L-Asparagine	Blue	Blue	Blue	Blue	Blue	Blue	
C4 L-Phenylalanine	Orange	Red	Orange	Orange	Green	Red	
D4 L-Serine	Blue	Blue	Orange	Blue	Blue	Blue	
E4 L-Threonine	Orange	Yellow	Blue	Blue	Blue	Yellow	Amin
F4 β -Hydroxyglycyl-L-glutam- ic acid	Red	Red	Orange	Orange	Red	Red	
G4 Phenylethylamine	Yellow	Red	Red	Red	Green	Yellow	
H4 Putrescine	Green	Green	Orange	Blue	Blue	Blue	

Note: Utilisation rates of carbon sources are expressed as a percentage of the respective AWCD. A five-level scale was used to indicate availability: red (<25 %); orange (25.1–50 %); yellow (50.1–75 %); green (75.1–100 %); blue (>100 %).

than the average in the sample plots for each depth. Other sources such as G1, H1, A2, C2, G2, C3 and G3 in SP1 had lower utilisation rate in the three depths, comprising less than 25 % of the average. Due to the higher metabolic activity in SP2, the lowest utilisation rate was registered only for D3 and G3 for the three depths. Other authors also reported higher utilisation of Tween 40 and Tween 80 in soils from different habitats such as mountain forests (Grigorova-Pesheva 2023), floodplains (Barros et al. 2023), tropical coastal lagoon (Behera et al. 2018). γ -Aminobutyric acid is accumulated by plants in response to abiotic stress factors (Sita and Kumar 2020) as it provides tolerance to salinity (Ma et al. 2018, Khanna et al. 2021) through improvements in growth, photosynthesis, enzymatic and non-enzymatic antioxidative defence mechanisms, and nitrogen metabolism (Ma et al. 2018, Salah et al. 2019).

Good metabolisation of L-Arginine, L-Asparagine and L-Serine was also reported by Barros et al. (2023). This could be related to the accumulation of soil organic matter containing nitrogen from different forms of amino acids, whose production and utilisation is related to their composition (Cao et al. 2021). Furtak et al. (2020) and Barros et al. (2023) also found that 2-hydroxybenzoic acid was not utilised during the flood periods in floodplains because it is difficult to degrade (Rutgers et al. 2016). Additionally, Onet et al. (2024) reported that higher utilisation rate of 2-hydroxybenzoic acid, glucose-1-phosphate and α -ketobutyric acid could indicate a decline in forest condition, while a higher utilisation rate of i-erythritol is a sign of both decline and decay, which was not observed in the current study. On the other hand, carbon sources such as D-galactonic acid γ -lac-

tone and L-Asparagine are also indicators of decay and decline (Onet et al. 2024), but in the current investigation they exhibited high overall utilisation rates. This means that additional investigations on forest condition should be done because these results could be an early warning for its deterioration. The Shannon diversity index, based on the utilisation rate of the 31 carbon sources was highest in SP2 for 0–10 cm depth (3.14). The lowest was calculated for SP1 10–20 cm depth (2.88). As a whole, the diversity index was higher in SP2 (on average 3.06 ± 0.07 for the three depths), while for SP1 it was 2.93 ± 0.07 . This confirms the different utilisation rate among the depths for SP2. The strong positive correlation ($r = 0.99$, $p < 0.01$) between the diversity and evenness indices proves that there are differences in the utilisation rate not in the number of the carbon sources. Similar results for the indices are reported for soil microbial communities in eucalyptus plantations of different age (Lan et al. 2019) and in different forests in Southwest China (Peng et al. 2021).

Statistical analysis showed that there was not a correlation between the metabolic activity and depth in both sample plots. Temperature ($r = -0.98$, $p < 0.01$ for SP1 and $r = -0.96$, $p < 0.01$ for SP2) and humus content ($r = -0.93$, $p < 0.01$ for SP1 and $r = -0.95$, $p < 0.01$ for SP2) correlated negatively with depth, and pH positively in SP2 ($r = 0.98$, $p < 0.01$). In SP1 there was a positive correlation between CA and pH ($r = 0.82$, $p < 0.01$), while in SP2 CGs' utilisation did not correlate with soil physico-chemical parameters.

Similarly, Grigorova-Pesheva (2023) did not identify correlation of soil acidity on the microbial activity, but there was a positive correlation with the soil organic content. Peng et al. (2021) also reported

that pH was not the main factor responsible for the microbial functional variation, but it was soil total nitrogen and available nitrogen. Overall, soil metabolic diversity depends on a complex of factors including soil properties in different forest types (Peng et al. 2021), vegetation type and its litter quality and quantity Dequiedt et al. (2011). Here, the observed differences in the soil metabolic activity between the two sample plots and also among the CSs and CGs may result from local conditions, related with the topography and microclimate peculiarity. Further soil research is needed for identifying the main driving factor for the differences in the metabolic activity also such related with seasonal dynamics of the soil microbiota.

Conclusions

Assessing soil microbial activity is an appropriate approach for understanding soil ecosystem function. Differences between the sample plots, despite having the same vegetation stand and similar soil types, indicate that the microbial metabolic profile is a sensitive indicator of local environmental influences. Weak utilisation of certain carbon substrates (2-hydroxybenzoic acid, glucose-1-phosphate, α -ketobutyric acid and i-erythritol) could indicate forest health, whereas higher utilisation of others (D-galactonic acid, γ -lactone and L-Asparagine) may serve as early-warning sign of forest decay and decline. These results demonstrate that functional indicators are reliable, as microorganisms respond quickly by adjusting their metabolism to even minor environmental changes. Additional analyses of temporal changes in the soil physiological profile are needed to enrich understanding of microbial activity and its relationship with vegetation stand

health.

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Ethics statement

All experimental procedures were conducted without the involvement of animals or human participants.

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Current volume increment of high-stemmed European beech stands in Petrohan Training and Experimental Forest Range

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Thematic field: Forest stand dynamics, growth and yield

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Abstract

The aim of this study is to determine the current volume increment of high-stemmed European beech (*Fagus sylvatica* L.) stands within the territory of Petrohan Training and Experimental Forest Range at elevations ranging from 1400 to 1600 m a.s.l. Permanent and temporary sample plots were established in pure, simple, relatively even-aged stands. Trees within the plots were measured using callipers, and several heights within each diameter class were taken to determine the standing volume. Increment cores from trees of varying diameter classes were taken using a Pressler increment borer to measure the current radial increment over the last ten years. The width of annual rings was measured using CooRecorder software. The current volume increment was estimated using two methods. The first method is based on the difference between the volume measured at the time of the study and the volume ten years ago, while the second method was based on growth tables. Comparison was made between the volume increment results obtained by the two methods. The results indicate that the increment values established in the present study considerably exceed (on average by 5–6 times) the increment levels derived from the growth tables at stand density 1.0. The highest increment rates (8.1 and 8.4 m³·ha⁻¹) were recorded in stands with a density above 1.0 (1.2–1.3). Equations derived using Gerhardt's formula for calculating the current increment were developed, which may be applied in beech forests within the enterprise's territory at the indicated altitudes.

Key words: Bulgarian mountain forests, *Fagus sylvatica*, increment cores, periodic annual increment, relative stocking, standing volume.

Introduction

Forests are a vital biological resource, providing timber as well as many other goods and ecosystem services. As a renewable resource and given its intensive utilisation and the growing demand for it, key objectives of forest management are its maintenance, conservation, and the maximisation of its productive potential.

However, studies concerning growth and productivity of European beech (*Fagus sylvatica* L.) are not unequivocal. According to some authors, in recent decades, European beech has increased the productivity of its stands (Pretzsch et al. 2014, Bosela et al. 2016, Kulla et al. 2023), thereby reinforcing its key importance in the mountainous regions of Europe even under changing climatic conditions. However, other studies have reported a decline in the growth rate and productivity of beech stands over the past decades (Geßler et al. 2007, Rohner et al. 2021, Martínez del Castillo et al. 2022, Levanic et al. 2023). The main factor contributing to the reduced productivity of beech forests is thought to be the changing climatic conditions, characterised by increasingly frequent drought periods.

Research conducted by Dimitrov (2019) in Bulgaria, in Western and Central Balkan Mountains, indicates that the growth of low-mountain beech forests is influenced primarily by the amount of precipitation, whereas the growth of high-mountain beech forests is strongly dependent on the temperature regime.

Tree growth has become one of the most important parameters for assessing both the response of individual trees and the overall condition of forests under changing environmental conditions (Spiecker et al. 1996, Dobbartin et al.

2013).

The growth of a forest stand is a complex biological process that is expressed through the increase in stand volume resulting from the annual increment of its constituent trees. Depending on the inventory indicator, the reference time period, and the calculation method used, several types of increment can be distinguished. In forestry, most significant are the current volume increment and the mean annual volume increment. The current increment is particularly important in forest management and silvicultural practices, as it relates to crucial aspects of forest production and utilisation.

This interest in increment dynamics is well justified. As forest management becomes increasingly intensive, the importance and necessity of accounting for the current volume increment has increased (Dimitrov 2011). Estimating this metric is crucial for evaluating the economic efficiency of both ongoing and planned silvicultural practices. Furthermore, volume increment serves as a key indicator of forest productivity. By analysing current increment trends, foresters can forecast stand development, assess stand condition, and determine the sustainable annual yield, thereby ensuring that ecological balance is maintained.

The increment and timber volume of a given stand are influenced by various factors. Among the most significant are species composition, site conditions, age, stand density, origin, climate, and the applied silvicultural practices (Dukhovnikov 1971, Bogdanov 1991, Mihov 2005, Tonchev et al. 2012). Each of these factors affects growth and productivity in specific ways.

A wide range of methods is available and commonly used in practice to estimate the current increment. These meth-

ods vary in accuracy and present distinct advantages and limitations.

The most widely used methods in forest management practice and scientific research for estimating the current increment include:

(1) single-time measurements through felling of sample trees or by extracting increment cores,

(2) re-measurement and long-term monitoring of permanent sample plots, and

(3) increment estimation based on growth and yield tables.

Re-measurement of permanent sample plots is considered one of the most reliable methods for determining increment in forestry. The obtained results are generally characterised by high accuracy and are close to the actual growth values. According to Tyurin (1938), the error of this method is within $\pm 10\%$, which is regarded as acceptable in forest research and practice.

Single-time measurements using sample tree felling or increment coring are also widely applied and highly precise methods for determining current increment. These approaches provide direct information on tree growth through the analysis of annual growth rings. Dimitrov (2011) summarises findings from Naumenko (1941), Moisenko and Murashko (1940), Babakin (1962) and others, noting that results obtained through sample tree felling closely correspond to those from re-measured permanent plots. Regarding increment cores, several authors, such as Tyurin (1938), Dzhurdzhu (1957), Dvoretzky (1960), Zagreev (1963), and Antanaitis (1969) and others consider that the error is within the range $\pm 12\text{--}15\%$, and in some cases even higher.

The use of growth and yield tables to

determine current increment is based on empirical models developed from observations of stand development under various site quality and age conditions. These tables provide reliable estimates when applied to uniform, even-aged stands that have not been subject to significant disturbances, and when regional growth tables specific to the geographic area are used. Under such conditions, yield tables can provide increment estimates with an error ranging between $\pm 10\text{--}15\%$ (Avery and Burkhardt 2002, Assmann 1970).

Therefore, the aim of this study is to determine the current volume increment of high-stemmed European beech (*Fagus sylvatica*) stands within the Petrohan Training and Experimental Forest Range at elevations of 1400–1600 m a.s.l., and to evaluate the obtained increment values in comparison with standard growth and yield table estimates.

Object and Methods

Object

The study was conducted on eight sample plots located within the territory of the Petrohan Training and Experimental Forest Range, situated at altitudes ranging from 1400 to 1600 metres above sea level (Fig. 1). Forest stands in the plots consist of pure, simple-structured, and relatively even-aged European beech stands. Most of the stands are found on south- and west-facing slopes, situated on gently to moderately inclined terrain on well-drained brown forest soils.

Detailed description of the sample plots, including their geographical and forest typological characteristics, is presented in Table 1.

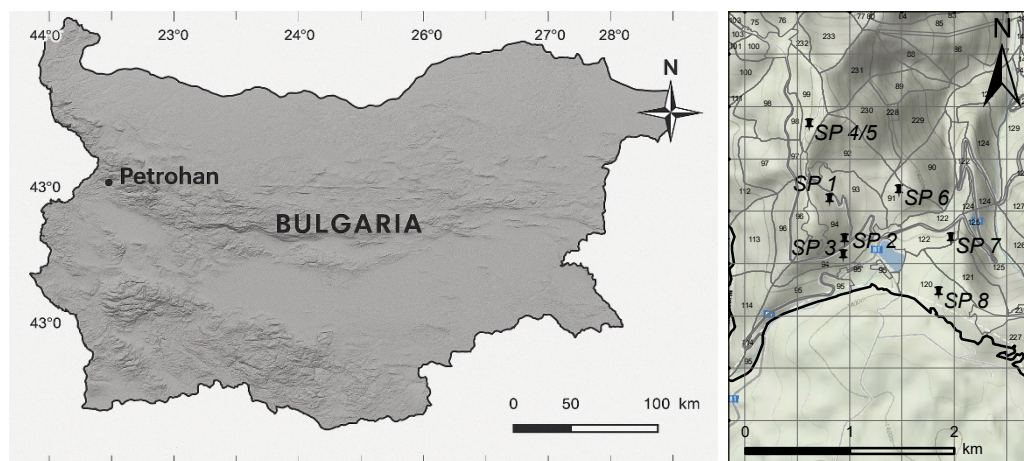


Fig. 1. Location of the study sites.

Table 1. Physico-geographical description of the experimental plots.

Sample plots	Area, ha	Latitude	Longitude	Elevation, m	Aspect	Slope, °	Soil type
SP1	0.40	43°7'33"	23°7'9"	1531	NE	12	Cambisol
SP2	0.20	43°7'21"	23°7'16"	1472	S	20	Cambisol
SP3	0.25	43°7'16"	23°7'16"	1439	S	17	Gleyic Cambisol
SP4	0.09	43°7'56"	23°6'59"	1606	W	13	Cambisol
SP5	0.09	43°7'56"	23°6'59"	1606	W	13	Cambisol
SP6	0.28	43°7'37"	23°7'38"	1437	SW	7	Cambisol
SP7	0.27	43°7'23"	23°8'1"	1420	W	22	Cambisol
SP8	0.27	43°7'6"	23°7'57"	1485	NW	18	Gleyic Cambisol

Methods

Tree diameters at breast height (DBH) were measured in all sample plots with an accuracy of ± 1 mm. Additionally, 2 to 4 tree heights were measured for each 2 cm diameter class to construct the DBH–height curve. Following the field measurements, key mensurational parameters were determined, including mean diameter, mean height, basal area, site index, and others. Based on the number of trees per diameter class, the following basal area components were calculated:

- 1) individual tree basal area,
- 2) total basal area, and

3) mean basal area, using the following standard forestry formulas (1), (2), and (3):

$$= \frac{\pi^2}{4}, \quad (1)$$

$$G = \sum (n_i \cdot g_i), \quad (2)$$

$$\bar{g} = \frac{G}{N}, \quad (3)$$

where: g – individual tree basal area, m^2 ; d – tree diameter at breast height, m; G – total basal area, m^2 ; n_i – number of trees in the i -th diameter class; g_i – basal area of a tree in the i -th diameter class; $\bar{g}$ – mean basal area, m^2 ; N – total number of trees in the sample plot.

The mean diameter of each plot was calculated using the formula (4):

$$\bar{d} = \sqrt{\frac{4 \cdot \bar{g}}{\pi}}. \quad (4)$$

The mean height of the forest stands was calculated using Lorey's formula (5):

$$\bar{H}_L = \frac{\sum (g_i \cdot h_i)}{\sum g_i}, \quad (5)$$

where: $h_1, h_2, h_3, \dots, h_n$ – arithmetic mean heights for each diameter class, m, $g_1, g_2, g_3, \dots, g_n$ – basal areas corresponding to each diameter class, m², n – number of diameter classes.

After determining the mean height and considering the updated mean stand age from the 2015 forestry management plan of the Petrohan Training and Experimental Forest Range (EFA 2015), the site quality (site index) of each stand was established using the site index table for seed-origin natural beech stands (Nedyalkov 1960 and Gerhardt 1923).

The stand relative stocking of each investigated stand was calculated as the ratio, according to formula (6), between the volume of the stand estimated in the current study and the volume of a normal stand of the same age and site index, based on yield tables for high-stemmed beech stands (Nedyalkov 1960 and Gerhardt 1923):

$$k = \frac{V_A}{V_{Tabl.}}, \quad (6)$$

where: k – relative stocking; V_A – current stand volume, m³·ha⁻¹; $V_{Tabl.}$ – volume of the normal (fully stocked) stand according to the yield tables, m³·ha⁻¹.

The standing volume of the sample plots was estimated using the software tool FET (Forestry Estimation Tool), developed in MS Excel by Eng. Evgeni Evangelov (Evangelov et al. 2012). This software is used for stem volume estima-

tion and assortment classification, based on mathematical models and coefficients designed to approximate the values provided in standard yield tables.

The input parameters required for the calculation of stand volume are the number of trees per diameter class and the mean height for each class. Heights for individual diameter classes were derived from height curves constructed separately for each stand and smoothed using a logarithmic function. Using the resulting logarithmic equation, the height of each tree was calculated based on its diameter.

The current volume increment was determined using two methods. The first method involved collecting increment cores at breast height with a Pressler increment borer, due to the absence of repeated measurements from permanent sample plots and the need to avoid destructive sampling through felling sample trees. According to Dimitrov (2011), it is sufficient to collect one to three samples per diameter class. In compliance with Ordinance No. 18 (2015) on the investigation of stand growth dynamics, a minimum of three sample trees should be collected for the three central diameter classes, two in the adjacent classes, and one for each subsequent class. The central diameter classes represent the middle diameter classes that include the majority of trees within a given stand. They encompass the class corresponding to the mean diameter and the two adjacent classes. This approach was adopted in the present study.

The width of annual rings on the increment cores was measured using the CoReRecorder and CDendro software (Maxwell and Larsson 2021) in the Department of Plant pathology and Chemistry at the University of Forestry, Sofia. The cores were scanned at a resolution exceeding 1200 dpi. The specialised software allows

measurement of radial increment with an accuracy of $\pm 1 \mu\text{m}$ ($\pm 0.001 \text{ mm}$).

The current volume increment using this method was calculated according to formula (7), based on the difference between the current volume (V_A) and the volume ten years earlier (V_{A-n}), divided by the time interval (n):

$$Z_v = \frac{V_A - V_{A-n}}{n} \quad (7)$$

To determine the stand volume ten years prior, the diameters and heights of the trees from ten years ago were used.

After determining the diameter increment over the past ten years from increment core analysis, the diameter of each tree ten years ago (d_{a-n}) was calculated by subtracting the diameter increment (Z_d) from the current diameter (d_a) of each tree in the sample plot. The diameter increment was calculated as twice the radial increment, and formula (8) can be expressed as follows:

$$d_{a-n} = d_a - Z_d \quad (8)$$

Height values for n years prior were calculated by applying a coefficient derived from growth tables. Specifically, the height corresponding to a given diameter from the height curve was multiplied by a coefficient equal to the ratio between the mean height at age n years ago ($\bar{H}_{L-n}$) and the mean height at current age ($\bar{H}_L$).

The second method for calculating the current volume increment is based on stand volume data derived from the growth tables of Nedyalkov and Gerhardt, with the application of formula (7) once more.

Based on the data from the present study, models were developed following Gerhardt's formulas – equation (9) for light-demanding tree species and equation (10) for shade-tolerant tree species. These equations incorporate the stand

volume as derived from growth and yield tables for the respective tree species, along with the stand relative stocking.

$$PAI = PAI_{Tabl.} \cdot (1.7 - 0.7 \cdot k) \cdot k, \quad (9)$$

$$PAI = PAI_{Tabl.} \cdot (2 - k) \cdot k, \quad (10)$$

where: PAI is periodic annual increment, $\text{m}^3 \cdot \text{ha}^{-1}$; $PAI_{Tabl.}$ – periodic annual increment, obtained from yield table, $\text{m}^3 \cdot \text{ha}^{-1}$; k – relative stocking.

In addition, the percentage of current volume increment ($P_V^{cur.}$) was calculated using Pressler formula (11), which is internationally recognised and widely used in global practice.

$$P_V^{cur.} = \frac{V_A - V_{A-n}}{V_A + V_{A-n}} \cdot \frac{200}{n}, \quad (11)$$

where: V_A – current stand volume, $\text{m}^3 \cdot \text{ha}^{-1}$, V_{A-n} – stand volume ten years ago, $\text{m}^3 \cdot \text{ha}^{-1}$, n – number of years.

Results

Table 2 presents the main dendrometric characteristics of the investigated beech stands. The studied stands range in age from 130 to 150 years and exhibit high growing stock levels, exceeding 500–600 $\text{m}^3 \cdot \text{ha}^{-1}$.

For comparative purposes, stand relative stocking and site index for each stand were determined using two sets of growth and yield tables: those for high-stemmed beech stands developed by Nedyalkov (1960), and the yield tables for beech stands by Gerhardt (1923).

Site indices and relative stocking derived from Nedyalkov's tables were consistently higher than those obtained using Gerhardt's tables. In terms of site index, the difference was equivalent to one site class for each stand, while for relative stocking, the average difference was 0.08.

Without engaging in a detailed com-

Table 2. Characteristics of stands in sample plots.

Sample plot	Average age, years	Average height, m	Site index*	Relative stocking*	Site index**	Relative stocking**	Average DBH, cm	Growing stock, m ³ ·ha ⁻¹
SP1	140	30.6	I	1.03	II	0.96	33.4	626
SP2	140	29.0	I	1.11	III	1.19	34.7	677
SP3	140	32.0	I	1.01	II	0.95	42.9	615
SP4	130	25.7	II	1.29	III	1.18	28.7	636
SP5	130	25.1	II	1.25	III	1.15	28.9	615
SP6	150	31.1	I	1.04	II	0.97	39.3	637
SP7	140	30.6	I	1.16	II	1.09	36.5	705
SP8	140	25.8	II	1.03	III	0.94	34.7	514

Note: * – Site index and relative stocking according to Nedyalkov's tables (1960), ** – Site index and relative stocking according to Gerhardt's tables (1923).

parison between the two sets of tables or their suitability for the specific site conditions of the study area, it can nevertheless be summarised that in half of the investigated stands (SP1, SP3, SP6, SP8) the relative stocking was close to the theoretical maximum value of 1.0 (ranging from 0.94 to 1.04). In the remaining stands (SP2, SP4, SP5, SP7), the relative stocking exceeded the theoretical maximum, with an average value of approximately 1.2 (ranging from 1.09 to 1.29).

Given that growth and yield tables represent stands with the highest possible growing stock – characterised by a relative stocking level of 1.0 – it may be concluded that half of the investigated stands are more 'fully stocked' with timber than

those in the reference tables, while the remaining half have growing stock levels comparable to them.

These findings, supported by the generally high site indices observed across the studied stands, suggest correspondingly high values of the Periodic Annual Increment (PAI). The results for PAI, as determined using the increment core method described above, are presented in Table 3. For comparative purposes, PAI values were also calculated under the assumption of full stocking ($k = 1.0$) using data from the yield tables of Nedyalkov (1960) and Gerhardt (1923).

Table 3 shows that the PAI values obtained through increment core analysis range from 4.9 m³·ha⁻¹ (SP8) to 8.4 m³·ha⁻¹ (SP4).

Table 3. Periodic annual increment (PAI) determined using increment cores and PAI according to yield tables for normal relative stocking ($k = 1.0$).

Method	Sample plot							
	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8
Periodic annual increment, m ³ ·ha ⁻¹								
Using increment cores	5.7	6.1	5.8	8.4	8.1	6.1	7.1	4.9
According to yield tables*	1.4	1.4	1.4	1.4	1.4	0.4	1.4	1.0
According to yield tables**	1.1	0.9	1.1	1.2	1.2	1.0	1.1	0.9

Note: * – Based on yield tables by Nedyalkov (1960), ** – Based on yield tables by Gerhardt (1923).

(SP4). The corresponding PAI values, derived from the yield tables of Nedyalkov and Gerhardt under the assumption of full stocking ($k = 1.0$), generally fall within the range of 0.9 to 1.4 $\text{m}^3 \cdot \text{ha}^{-1}$, with the exception of SP6, where the increment based on Nedyalkov's tables is only 0.4 $\text{m}^3 \cdot \text{ha}^{-1}$. This relatively low value for SP6 is attributed to the minimal increase in growing stock – only 4 $\text{m}^3 \cdot \text{ha}^{-1}$ – between the ages of 140 and 150 years.

Based on the results, the increment values obtained in this study are significantly higher (on average, 5–6 times higher) than those derived by fully stocked yield tables. At the same time, the actual relative stocking of the investigated stands was at or above 1.0, with the highest PAI values recorded in SP4 (8.4 $\text{m}^3 \cdot \text{ha}^{-1}$) and SP5 (8.1 $\text{m}^3 \cdot \text{ha}^{-1}$), where the relative stocking reached approximately 1.2 to 1.3.

Table 4 presents the calculated confidence intervals for the increment cores for each sample plot.

The mean radial increments from increment cores in the eight sample plots (SP1–SP8) ranged from 1.37 mm to 2.04 mm per year, reflecting local differences in growth conditions and individual tree characteristics. The calculated 95% confidence intervals indicate substantial variability across plots, with interval widths ranging from 0.5 mm to 0.8 mm,

attributable to trees of varying social status (dominant vs. suppressed).

Table 5 presents the percentage increment values calculated using equation (11). The increment obtained from increment cores in this study ranges from 0.94 % to 1.41 %, whereas the values derived from the yield tables mostly range between 0.15 % and 0.29 %, with the exception of SP6 due to the minimal increase in growing stock observed in that plot.

According to Dimitrov (2000), the highest increment levels are achieved at a relative stocking density between 0.8 and 1.0. This is partially supported by Gerhardt's formulas – equation (9) for light-demanding tree species and equation (10) for shade-tolerant tree species – where any deviation of the relative stocking factor from 1.0 (whether above or below) reduces the calculated increment.

In this study, the coefficient used in equation (10) was recalculated based on the increment values obtained from increment cores and compared against the increments derived from the yield tables of Nedyalkov – equation (12) and Gerhardt – equation (13).

$$PAI = PAI_{Tabl.} \cdot (5.46 - k) \cdot k, \quad (12)$$

$$PAI = PAI_{Tabl.} \cdot (6.48 - k) \cdot k. \quad (13)$$

Table 6 presents the recalculated increment values for the sample plots

Table 4. Confidence intervals of increment cores

Sample plots	<i>n</i>	Mean increment, mm	SD	95% CI – lower bound	95% CI – upper bound
SP1	31	1.462	0.920	1.124	1.799
SP2	27	1.370	0.693	1.096	1.645
SP3	29	1.913	0.875	1.580	2.246
SP4	30	1.470	0.857	1.150	1.789
SP5	31	1.478	0.849	1.167	1.790
SP6	30	2.042	1.079	1.647	2.437
SP7	30	1.556	0.924	1.217	1.895
SP8	29	1.436	0.870	1.103	1.770

Table 5. Periodic annual increment (PAI), determined using increment cores, and PAI according to yield tables for normal relative stocking ($k = 1.0$).

Method	Sample plot							
	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8
	Periodic annual increment, %							
Using increment cores	0.95	0.94	0.99	1.41	1.41	1.01	1.06	1.00
According to yield tables*	0.23	0.23	0.23	0.29	0.29	0.07	0.23	0.20
According to yield tables**	0.17	0.17	0.17	0.23	0.23	0.15	0.17	0.17

Note: * – Based on yield tables by Nedyalkov (1960), ** – Based on yield tables by Gerhardt (1923).

Table 6. Periodic annual increment (PAI), determined using increment cores and PAI, determined using yield tables and Gerhardt's formula with coefficients of current study.

Method	Sample plot							
	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8
	Periodic annual increment (PAI), m ³ ·ha ⁻¹							
Using increment cores	5.7	6.1	5.8	8.4	8.1	6.1	4.9	7.1
Using yield tables*	6.4	6.8	6.3	7.6	7.4	1.8	4.6	7.0
Using yield tables**	6.2	6.2	6.1	8.0	7.8	5.7	5.0	6.9
	Deviation of PAI – using yield tables, versus PAI – using increment cores, %							
Using yield tables*	12.2	11.2	8.7	-10.0	-8.8	-69.8	-7.1	-1.6
Using yield tables**	9.4	2.4	5.9	-4.3	-3.4	-6.9	2.0	-3.2

Note: * – Based on yield tables by Nedyalkov (1960), ** – Based on yield tables by Gerhardt (1923), modified with coefficients derived in the present study.

based on yield table data and equations (12) and (13). The percentage deviations of these values from the increment determined using the increment core method were also calculated. From the table, it can be observed that the increment estimates derived from the growth tables and corresponding equations deviate by no more than $\pm 12\%$ from the increment values established through increment core sampling. An exception is again found in SP6, where the value obtained using Nedyalkov's tables shows a larger deviation. As previously discussed, this discrepancy is due to the minimal increase in growing stock observed between age classes, which significantly affects the increment estimate in this stand.

The evaluation of the proposed empirical relationships was performed by comparing the increment values obtained from increment cores with the PAI values calculated using equations (12) and (13). Standard modelling accuracy indicators were employed for this purpose, including the correlation coefficient (R), coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE) (Table 7).

Table 7. Statistical evaluation of results obtained from the derived equations.

Method	R	R ²	RMSE	MAE
Nedyalkov (1960)	0.88	0.77	1.48	1.10
Gerhardt (1923)	0.97	0.93	0.33	0.24

The high correlation coefficients ($R = 0.88$ and $R = 0.97$) indicate a strong linear relationship between compared PAI results. The coefficients of determination ($R^2 = 0.77$ and $R^2 = 0.93$) show that approximately 77 % of the variability in PAI is explained by equation (12), and around 93 % by equation (13). It should be noted that SP6 was excluded when calculating R for equation (12) due to the previously mentioned circumstances. The low values of RMSE and MAE confirm the accuracy and low approximation error, indicating that the models adequately describe the relationship between the variables. Therefore, proposed equations demonstrate good predictive capability and can be used for estimating PAI under the respective conditions.

The comparison between PAI values obtained from increment cores and those estimated using the stand volume growth tables (Nedyalkov and Gerhardt) showed relatively small differences between the methods (Table 7). To assess whether these differences were statistically significant, a paired t -test was applied. The results indicated no statistically significant differences between the methods ($t = -0.93$, $p = 0.38$ for Nedyalkov; $t = -0.31$, $p = 0.76$ for Gerhardt). This suggests that both approaches based on growth tables provide comparable estimates of periodic annual increment relative to those obtained from increment cores. The observed deviations may be attributed to random variation rather than systematic differences between the methods.

Discussion

In a broader context, obtained results align with trends reported by several authors (Gatti et al. 2019, Hilmers et al. 2019,

Pretzsch et al. 2020), according to which beech forests in montane and submontane zones can maintain or even increase their growth under moderate climatic conditions. Conversely, in lowland and Mediterranean regions (Piovesan et al. 2008, Jump et al. 2009), warming and drought lead to reduced productivity. In this sense, stands examined in this study, located on mid-mountain, well-watered sites, exhibit high productivity in the context of climate change.

Similar trends of accelerated growth have been documented for Central Europe. In a study by Hans Pretzsch et al. (2014), it was found that tree species such as European beech and Norway spruce have shown an increase in growth of 32–77 % compared to 1960, while volume increment increased by 10–30 % – an effect associated with a longer growing season and higher temperatures.

Poryazov (2009) developed local growth tables for high-stem beech stands in the Petrohan Forest Management Unit and compared them with the tables of Gerhardt (1930), Wiedemann (1931), Armasescu et al. (1954) (cited in Popescu et al. 1957), and Nedyalkov (1960). He found that his locally developed growth tables had the highest accuracy, followed by those of Gerhardt, Armasescu, and Nedyalkov, while Wiedemann's tables were least accurate. Poryazov reported current volume increment values approximately three times higher than those obtained using Nedyalkov's growth tables. Our results approach those of Poryazov, leading to the conclusion that local models more accurately reflect the actual productivity of stands under present conditions.

The proposed empirical relationships (equations 12 and 13) show relatively high degree of agreement with experimental data ($R^2 = 0.77$ – 0.93), indicating their re-

liability under local conditions, with higher accuracy achieved using the equation based on Gerhardt's tables. Nonetheless, the limited number of sample plots in the present study renders the results preliminary, preventing a full representation of the spatial variability of beech stands across the entire region. To enhance the validity of the conclusions, future research covering a wider range of conditions and a larger number of sample plots is required.

Conclusions

In the investigated high-stemmed beech stands, located at altitudes between 1400 and 1600 m a.s.l. within the territory of Petrohan Training and Experimental Forest Range, high levels of growing stock ($515\text{--}705\text{ m}^3\cdot\text{ha}^{-1}$), relative stocking ($0.9\text{--}1.3$), and Periodic Annual Increment (PAI, $4.9\text{--}8.4\text{ m}^3\cdot\text{ha}^{-1}$) were recorded. The increment values obtained through the increment core method exceeded those derived from the standard yield tables for high-stemmed beech stands by Nedyalkov (1960) and Gerhardt (1923) by a factor of approximately five to six on average. Highest increment levels (8.1 and $8.4\text{ m}^3\cdot\text{ha}^{-1}$) were observed in stands with relative stocking exceeding 1.0 (between 1.2 and 1.3).

Since Gerhardt's formulas yield maximum increment values at a relative stocking of 1.0, the coefficient in the equation for shade-tolerant species was recalculated based on the actual increment values observed in this study.

In conclusion, the present study confirms that high-stem beech stands in the Western Stara Planina are characterised by growth and productivity levels significantly exceeding those presented in the Nedyalkov and Gerhardt growth tables

used for comparison. The results emphasise the need for implementing local growth and increment models that accurately reflect the actual productivity of beech forests and support sustainable forest management in the mountainous regions of Bulgaria.

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Weed monitoring in three sub montane regions in Southern Bulgaria

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Abstract

The study was conducted on areas where the conservation agricultural systems were applied (No-Till). A crop rotation system of winter oilseed rape and winter wheat has been maintained for the past five years. The present research was carried out over two growing seasons (2019/2020 and 2020/2021). The absence of rotation and differences in the herbicide application, as well as the presence of the same species in the years of the survey, can be considered as the first signs requiring the conduct of annual regular surveys according to the methodology. Such monitoring is essential for the detection of herbicide-resistant weed species. The weed flora monitoring was performed in three agricultural locations: the town of Brezovo and the villages of Streltsi and Varben. A total of 54 weed species were identified, belonging to 22 botanical families, with a ratio of 89:11 (monocarpic : polycarpic). They were assigned to eleven biological groups, comprising 43 annual (monocarpic) and 10 perennial (polycarpic) species. Among the annuals, 6 were monocotyledonous, 36 dicotyledonous, and 1 bulbous species. The family *Poaceae* was the most abundant in the surveyed areas, represented by 9 species distributed across four biological groups: annual species, from which: winter spring – *Apera spica-venti* (L.) P.Beauv.; overwintering – *Bromus arvensis* L.; late spring – *Setaria viridis* (L.) P.Beauv., *Echinochloa crus-galli* (L.) P.Beauv., *Setaria glauca* (L.) P.Beauv.; early spring – *Avena fatua* L., *Lolium temulentum* L.; and perennials (rhizomatous weeds) – *Sorghum halepense* (L.) Pers. and *Cynodon dactylon* (L.) Pers. The aim of this research was to characterise the weed flora in crop production areas under the crop rotation system of winter oilseed rape and winter wheat.

Key words: crop rotation, weed infestation, weed reporting.

Introduction

In Bulgaria, according to the data of the Ministry of Agriculture and Food (MAF 2025), oilseed rape is cultivated on 66,343 ha of agricultural land, with a total production of 158,134 t. Weeds occurring alongside the cultivated crop are among the most limiting factors for optimal development and productivity of the plants (Tonev et al. 2019).

In agriculture, approximately 34 % of the crop losses are due to uncontrolled weeds, while the yield reduction caused by pests is estimated at 16–18 % (Oerke 2005). Competition between crop and weeds in the field may also reduce the seed oil content (Moradi et al. 2020).

Other studies also reported that weed infestation in oilseed rape can reduce the yields by 50 %, while the quality of production is likewise diminished (Blackshaw et al. 2002, Konstantinović et al. 2007). In this regard, to obtain high- and good-quality yields from oilseed rape, timely and efficient weed control should be performed (Maataoui et al. 2003, Hamzei et al. 2010, Pavlović et al. 2015, Tonev et al. 2019).

Reporting and identifying weed flora in cropping systems is crucial for the effective weed control (Norsworthy et al. 2013). Studies, such as that conducted by Haq et al. (2023), which investigated weed infestation across five different agro-ecosystems in the Indian Western Himalayas (paddy, maize, mustard, apple, and vegetable), provide valuable guidance formulating the most appropriate weed management strategies in the region.

Weed monitoring from 2010 to 2019 was conducted by Tonev et al. (2021). The study found that the most commonly occurring weeds in fields sown with oilseed rape in Bulgaria were *Raphanus raphanistrum* L. and *Sinapis arvensis* L.

In other areas, species, such as *Papaver rhoeas* L., *Lolium* spp., and *Avena fatua* L., were recorded. Parasitic weeds of the genus *Phelipanche* were primarily found in the southern regions of the country.

Weed species composition shows variation depending on the latitude at which the crop is grown. Studies in Australia have shown that *Lolium rigidum* Gaudin, *Vulpia myuros* (L.) C.C.Gmel., and *Avena fatua* are the most abundant weeds in oilseed rape (Lemerle et al. 2001).

In Bulgaria, oilseed rape is typically followed by winter wheat in the crop rotation. When sown after winter wheat, the highest seed yield of oilseed rape has been recorded (Neshev 2022).

Winter wheat (*Triticum aestivum* L.) is the main crop plant in Bulgaria. One of the factors limiting its development is annual weed infestation (Yanev et al. 2014). Weeds are strong competitors with wheat for nutrients, water, space, and light. They can also cause indirect damage, as many species serve as hosts for harmful insects and diseases (Kalinova et al. 2012). Some weed species, such as *Cirsium arvense* (L.) Scop., *Consolida regalis* (L.) Gray, *Polygonum aviculare* L., *Consolida orientalis* (L.) Schur, *Convolvulus arvensis* L., *Centaurea cyanus* L., *Erodium cicutarium* (L.) L'Hér., and *Bifora radians* (Bieb.) W.D.J.Koch, have become an increasing problem in wheat (Susuri et al. 2001, Mehmeti et al. 2009, Mehmeti and Demaj 2010). As a result of weed-crop competition, wheat growth is weakened, which negatively affects grain yields (Al-Mafrajy 2018, Hammood and Safi 2018, Garakishi 2020). Yield losses due to weeds have been reported (Oerke and Dehne 2004, Dangwal et al. 2010), and high weed infestation in wheat fields can reduce yields by more than 70 % (Atanasova and Zarkov 2002).

Information on weed flora in the sub montane regions in Southern Bulgaria is limited. Therefore, the aim of the present study was to characterise the weed flora in crop production areas under the crop rotation system of winter oilseed rape and winter wheat, cultivated using no-till methods, in the lands of the town of Brezovo and the villages of Streltsi and Varben.

Materials and Methods

Monitored areas

The study was conducted in the Central Sredna Gora mountain region. Three locations were monitored for the purposes of the study: the town of Brezovo (42°20'42" N, 25°04'34" E) and the villages of Streltsi (42°23'48" N, 24°54'45" E) and Varben (42°25'10" N, 24°57'53" E), all situated in South-Central Bulgaria.

Crop plants and weed management techniques

The following crops were cultivated, and the corresponding weed management practices were implemented during the study period:

1. Winter oilseed rape (2019/2020) was sown at a density of 350,000 plants per hectare, using the hybrid 'In Vigor'.

2. Winter wheat (2020/2021) was sown at a density of 550,000 plants per hectare, using the variety 'Zemela'.

Twenty-one days before sowing, weeds were treated with 3.5 L·ha⁻¹ Round-up Classic Pro (360 g·L⁻¹ glyphosate).

For oilseed rape at the crop growth stage of 2–4 true leaves, the herbicide product Select Super 120 EC (120 g·L⁻¹ clethodim) at a rate of 1.6 L·ha⁻¹ was applied.

After sowing, but before germination of winter wheat and the weeds, the soil herbicide Konstel (25 g·L⁻¹ diflufenican + 400 g·L⁻¹ chlorotoluron) at a rate of 4.0 L·ha⁻¹ was sprayed.

Weed monitoring

The weed monitoring was performed by the quantitative method as described by Dimitrova et al. (2004). Weeds in both experimental years (2019/2020 and 2020/2021) were counted in square sample plots at five locations where herbicidal treatments were not applied for the purposes of the survey. Weed infestation was recorded by identifying species and their composition by using the 'Colour atlas of the 100 most economically important weeds in Bulgaria' (Tonev 2000). All weed species present were recorded during each survey.

The weed monitoring in the oilseed rape fields was conducted in the following times:

- Immediately before the application of the total herbicide;
- Immediately before sowing the crop;
- In the crop growth stage beginning of germination, appearance of the first cotyledons, before applying the foliar grass weed-controlling herbicide Select Super 120 EC;
- Second assessment during the vegetation – 20 days after spraying of Select Super 120.

The weed monitoring in the winter wheat fields was performed in the following periods:

- Immediately before applying the total herbicide;
- Immediately before sowing the crop;
- First reporting at 2–4 leaf stage of the crop;
- Second assessment – at the early til-

lering stage of the crop;

- Third assessment – at the advanced tillering stage of the crop.

Statistical analyses of the collected data

The statistical analysis of the results was conducted using one-way ANOVA, followed by Duncan's Multiple Range Test for post hoc comparisons, performed with the SPSS software package.

Results and Discussion

Characterisation of the weed flora in the study areas

According to a study performed by Fried et al. (2008), the major differences in

weed composition between the examined fields were associated with human management factors, the current crop, and its preceding crop.

For better understanding the results from the present research, the weed infestation data are presented as averages for the two experimental years, as total summarised for the crop rotation unit oil-seed rape – winter wheat. Weed species are shown collectively for all observation periods. The species composition of the weeds is presented in Table 1.

In quantitative terms, the diversity of annual weed species differed among the three study areas. Highest number of species was recorded in the region of Streltsi (30 species), followed by Varben (21 species) and Brezovo (15 species).

Interesting results were obtained from the analysis of perennial species across

Table 1. Average annual weed species recorded in the three study regions for both crops.

Biological group	Weed name	Brezovo	Varben	Streltsi	Total
Biannual weeds	<i>Malva sylvestris</i> L.	-	1	1	2
Ephemerals	<i>Veronica hederifolia</i> (L.)	-	-	1	1
	<i>Arabidopsis thaliana</i> (L.) Heynh.	1	-	-	1
	<i>Stellaria media</i> (L.) Cyr.	-	-	1	1
	<i>Myosotis stricta</i> L.	-	-	1	1
	<i>Fumaria officinalis</i> L.	-	1	-	1
	<i>Lamium amplexicaule</i> L.	1	1	1	3
	<i>Lamium purpureum</i> L.	-	-	1	1
	Ephemerals total	2 ^b	2 ^b	5 ^a	9
Winter – spring	<i>Arabidopsis thaliana</i>	-	-	1	1
	<i>Apera spica-venti</i> (L.) P.Beauv.	1	-	-	1
	<i>Vicia</i> spp.	-	1	1	2
	<i>Echinochloa crus-galli</i> (L.) P.Beauv.	-	-	1	1
	<i>Matricaria chamomilla</i> L.	-	-	1	1
	<i>Geranium molle</i> (L.)	-	1	1	2
	<i>Capsella bursa-pastoris</i> (L.) Medik.	-	1	1	2
	<i>Myosotis arvensis</i> L.	-	1	-	1
	<i>Papaver rhoeas</i> L.	1	-	-	1

Biological group	Weed name	Brezovo	Varben	Streltsi	Total
	<i>Anthemis arvensis</i> L.	-	1	-	1
	<i>Viola</i> spp.	-	1	1	2
	<i>Erodium cicutarium</i> (L.) L'Hér.	-	1	1	2
Winter-spring total		2 ^b	7 ^a	8 ^a	17
Overwintering		1	-	1	2
Overwintering total		1 ^a	0 ^b	1 ^a	2
Late spring	<i>Abutilon theophrasti</i> Medik.	-	1	1	2
	<i>Chenopodium album</i> L.	-	1	1	2
	<i>Sonchus oleraceus</i> L.	-	1	1	2
	<i>Setaria viridis</i> (L.) P.Beauv.	-	1	1	2
	<i>Conyza canadensis</i> (L.) Cronquist	-	-	1	1
	<i>Lactuca serriola</i> L.	-	1	1	2
	<i>Sonchus</i> spp.	-	-	1	1
	<i>Amaranthus retroflexus</i> L.	1	1	1	3
	<i>Poligonum aviculare</i> L.	1	-	1	2
	<i>Persicaria lapathifolia</i> (L.) Delarbre	1	-	-	1
	<i>Anagallis arvensis</i> L.	-	1	-	1
	<i>Amaranthus blitoides</i> L.	1	-	-	1
	<i>Xanthium strumarium</i> L.	-	1	1	2
	<i>Setaria glauca</i> (L.) P.Beauv.	1	-	-	1
	<i>Portulaca oleracea</i> L.	-	1	1	2
	<i>Solanum nigrum</i> L.	1	1	1	3
Late spring total		6 ^c	10 ^b	12 ^a	28
Early spring	<i>Avena fatua</i> L.	1	-	-	1
	<i>Bifora radians</i> (Bieb.) W.D.J.Koch	1	-	1	2
	<i>Spergula arvensis</i> L.	-	1	-	1
	<i>Galium aparine</i> L.	1	-	1	2
	<i>Lolium temulentum</i> L.	1	-	-	1
	<i>Poligonum convolvulus</i> L.	-	-	1	1
Early spring total		4 ^a	1 ^c	3 ^b	8
Annual weeds total		15 ^c	21 ^b	30 ^a	66

Note: Means with different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

the study sites. *Sorghum halepense* was found only in the production areas around Brezovo, whereas *Cynodon dactylon* was found mainly in the lands of Varben and Streltsi. Different pattern was observed for root-sprouting species: *Cirsium ar-*

vense and *Convolvulus arvensis* were found in the three examined areas. Bulbous species, as well as perennials with spindle-shaped roots, were found only in Varben and Streltsi. The obtained data are presented in Table 2.

Table 2. Average perennial weed species recorded in the three study regions for both crops.

Biological group	Weed name	Brezovo	Varben	Streltsi
Rhizomatous	<i>Sorghum halepense</i> (L.) Pers.	1	-	-
	<i>Cynodon dactylon</i> (L.) Pers.	-	1	1
Rhizomes total		1	1	1
Root-sprouting	<i>Cirsium arvense</i> (L.) Scop.	1	1	1
	<i>Convolvulus arvensis</i> L.	1	1	1
Root-sprouting total		2 ^a	2 ^a	2 ^a
Bulbous	<i>Muscari</i> spp.	-	1	1
Bulbous total		0	1	1
Spindle-rooted	<i>Trifolium</i> spp.	-	1	1
	<i>Lotus corniculatus</i> L.	-	1	1
	<i>Rumex acetosella</i> L.	-	-	1
	<i>Trifolium arvense</i> L.	-	-	1
	<i>Trifolium pratense</i> L.	-	1	1
Spindle-rooted total		0 ^c	3 ^b	5 ^a
Perennial weeds total		3 ^a	7 ^a	9 ^a

Note: Means with different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

Characterisation of the weed species

The total weed infestation reported in the present study is presented in Figure 1. Across the three study sites (the town of Brezovo and the villages of Streltsi and Varben), a total of 54 weed species, belonging to 22 botanical families, were documented. These comprised 89 % monocarpic, 9 % polycarpic and 2 % parasitic species. The recorded weeds were distributed across eleven biological groups.

Based on their lifespan, the species were classified as 43 annual (monocarpic) and 10 perennial (polycarpic) species. The annual species included 6 monocotyledonous and 36 dicotyledonous species, as well as one bulbous species.

Weed infestation varies across different regions. According to Salimi and Sajedi (2005), in Tehran Province, Iran, the most widespread weeds in oilseed rape were *Descurainia sophia* (L.) Webb ex

Prantl, *Cardaria draba* (L.) Desv., *Rapistrum rugosum* (L.) All., *Goldbachia laevigata* (M.Bieb.) DC., *Erysimum rependum* L., *Capsella bursa-pastoris* (L.) Medik., *Lamium amplexicaule* L., *Malva neglecta* Wallr., *Veronica persica* Poir., *Sonchus* spp., *Galium tricornutum* Dandy, *Vicia villosa* Roth, *Silene conoidea* L., *Fumaria officinalis* L., *Convolvulus arvensis*, *Avena ludoviciana* Durieu, *Bromus tectorum* L., *Euphorbia helioscopia* L. and *Muscari neglectum* Guss.

In Germany, the main weed infestation in winter oilseed rape were caused by *Matricaria* spp., *Viola arvensis* L., *Capsella bursa-pastoris*, *Stellaria media* (L.) Cyr., and *Thlaspi arvense* L. (Hanzlik et al. 2010).

In areas of north-east Scotland, the most frequently recorded weed species were *Poa annua* L., *Stellaria media*, *Viola arvensis*, *Capsella bursa-pastoris* and *Fumaria officinalis* (Gillian et al. 1992).

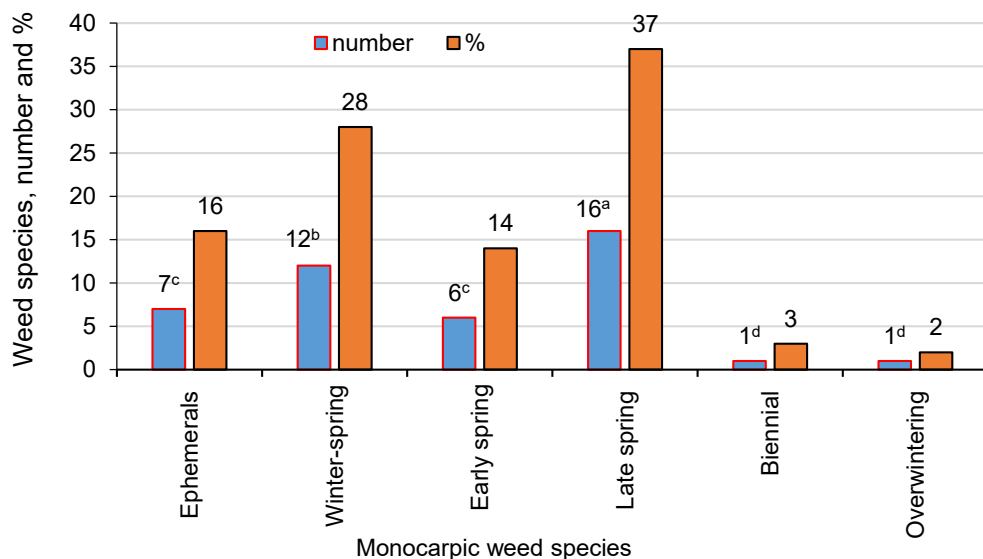


Fig. 1. Total distribution of annual (monocarpic) weed species.

Note: Means with different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

In Poland, within the Lublin region, the weeds that infested oilseed rape to the greatest extent were *Matricaria maritima* (L.) Rauschert, *Anthemis arvensis* L., *Viola arvensis*, *Capsella bursa-pastoris*, *Centaurea cyanus* and *Agropyron repens* (L.) Gould (Kapeluszny 2005).

In a trial conducted by Gawęda et al. (2018) in Poland, if winter wheat was sown after soybean the most frequently found weed species was *Avena fatua* L., whereas *Viola arvensis* was most abundant when wheat was sown after oilseed rape.

The results from an experiment conducted by Keshavarzi (2015) showed that 14 plant species, representing 7 families, were recorded as weeds. The families of greatest importance were Gramineae, Rubiaceae, Convolvulaceae, Fabaceae, Polygonaceae, Apiaceae and Caryophyllaceae. The most abundant broadleaf

weeds were *Galium tricornutum*, *Turgenia latifolia* (L.) Hoffm., *Vaccaria grandiflora* (Bertol.) Hook.f. & Thompson, and *Silene conoidea*. The most frequently recorded grass weeds were *Hordeum spontaneum* C.Koch and *Sorghum halepense* (L.) Pers.

Highest density (37 %) was recorded for late spring weeds. This group included two grass species from the family Poaceae – green bristle grass (*Setaria viridis*) and yellow bristle grass (*S. glauca*) – as well as fourteen dicotyledonous species belonging to nine families: Primulaceae – scarlet pimpernel (*Anagallis arvensis* L.); Solanaceae – black nightshade (*Solanum nigrum* L.); Polygonaceae – common knotgrass (*Polygonum aviculare*) and pale persicaria (*Persicaria lapathifolia* (L.) Delarbre); Chenopodiaceae – lamb's quarters (*Chenopodium album* L.); Malvaceae – velvetleaf (*Abutilon theophrasti* Medik.);

Asteraceae – sow thistle (*Sonchus oleraceus* L.), Canadian fleabane (*Conyza canadensis* (L.) Cronquist), prickly lettuce (*Lactuca serriola* L.), *Sonchus* spp., and common cocklebur (*Xanthium strumarium* L.); Amaranthaceae – common amaranth (*Amaranthus retroflexus* L.) and mat amaranth (*Amaranthus blitoides* S.Wats.); Portulacaceae – common purslane (*Portulaca oleracea* L.).

The percentage distribution of species by botanical family within the group of late spring weeds is presented in Figure 2.

Representatives of winter-spring species constituted 28 % of the weed association. The ratio of mono- to dicotyledons was 18:82 %. Species of the families Poaceae, Asteraceae and Brassicaceae each accounted for an equal share of 18%. These included wind bent grass (*Apera spica-venti* (L.) P.Beauv.) and barnyard grass (*Echinochloa crus-galli* (L.) P.Beauv.) from Poaceae; chamomile

(*Matricaria chamomilla* L.) and corn chamomile (*Anthemis arvensis*) from Asteraceae; and thale cress (*Arabidopsis thaliana* L.) and Shepherd's purse (*Capsella bursa-pastoris*) from Brassicaceae. Families represented with 9.09 % each were Boraginaceae – forget-me-not (*Myosotis arvensis* L.), Fabaceae – *Vicia* spp., Geraniaceae – dove's-foot cranesbill (*Geranium molle* L.), Papaveraceae – common poppy (*Papaver rhoeas*) and Violaceae – field pansy (*Viola* spp.).

The percentage distribution of species by botanical family within the group of early spring weeds is presented in Figure 3.

Representatives of the ephemeral group account for 16 % of the weed association, comprising seven botanical species (Fig. 4).

The identified ephemeral species were: small-flowered forget-me-not (*Myosotis stricta* L.), ivy-leaved speedwell (*Veronica hederifolia* L.), chickweed (*Stellaria*

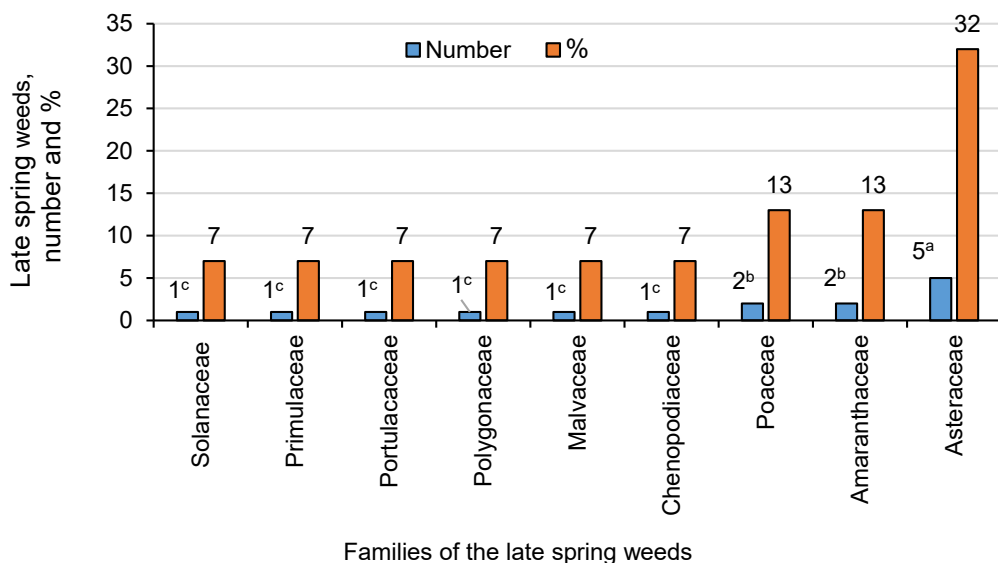


Fig. 2. Percentage distribution of late spring weed species by botanical family.

Note: Means with different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

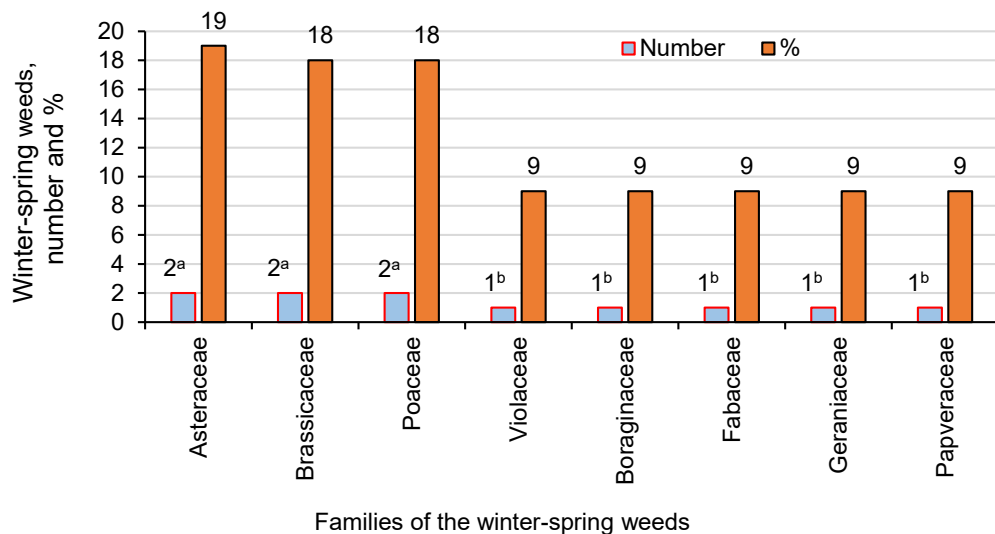


Fig. 3. Percentage distribution of species by botanical family within the group of winter-spring weeds.

Note: Means with different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

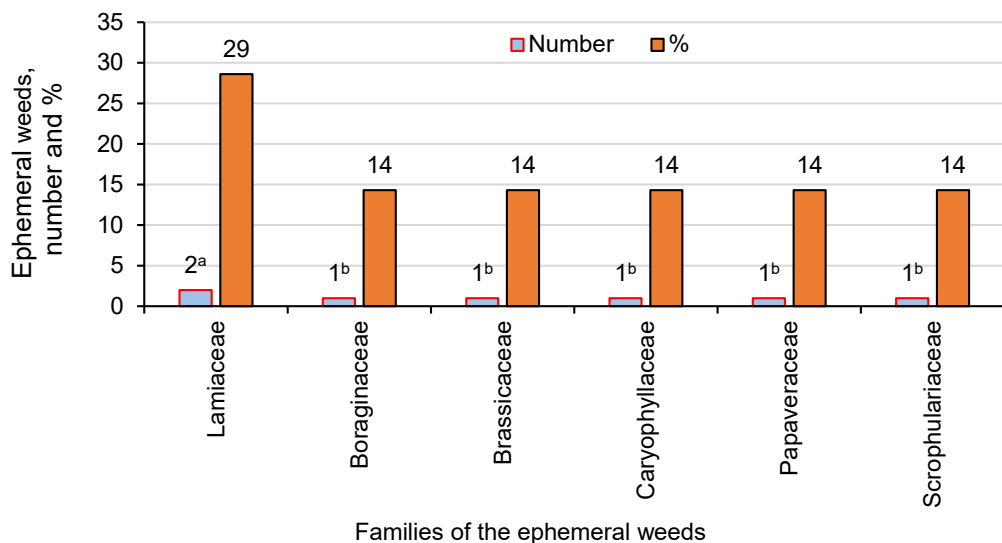


Fig. 4. Distribution of ephemeral weed species across botanical families.

Note: Means with different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

media), thale cress (*Arabidopsis thaliana* (L.) Heynh.), common fumitory (*Fumaria officinalis*), henbit dead-nettle (*Lamium amplexicaule*) and red dead-nettle (*Lamium purpureum* L.). These species were distributed across six botanical families as follows: Lamiaceae – 29 %, Boraginaceae – 14 %, Brassicaceae – 14 %, Caryophyllaceae – 14 %, Papaveraceae – 14 % and Scrophulariaceae – 14 %.

The identified early spring species were wild oat (*Avena fatua*), darnel (*Loium temulentum* L.), cleavers (*Galium aparine* L.), corn spurry (*Spergula arvensis* L.), wild buckwheat (*Polygonum convolvulus* L.) and wild bishop's weed (*Bifora radians*). These species were distributed across five botanical families, as follows: Poaceae – 32 %, Rubiaceae – 17 %, Caryophyllaceae – 17 %, Polygonaceae – 17 %, and Apiaceae – 17 % (Fig. 5).

Lowest shares were recorded for biennial and winter weeds, each represented

by a single species: Malvaceae – mallow (*Malva sylvestris* L.) and Poaceae – field brome (*Bromus arvensis* L.).

The distribution of perennial (polycarpic) weed species is presented in Figure 6. Atypical for most cultivated areas, the highest density was recorded for spindle-rooted species, which accounted for 50 % of the perennial weed association. This group comprised a total of five species distributed across two families. The Fabaceae family included clover (*Trifolium* spp.), bird's-foot trefoil (*Lotus corniculatus* L.) and field clover (*Trifolium arvense* L.), while the Polygonaceae family was represented by field sorrel (*Rumex acetosella* L.).

The most persistent groups of perennial weeds – root-sprouting and rhizome species – were present in a 1:1 ratio and together occupy 20 % of the weed community.

The rhizome species were Johnson

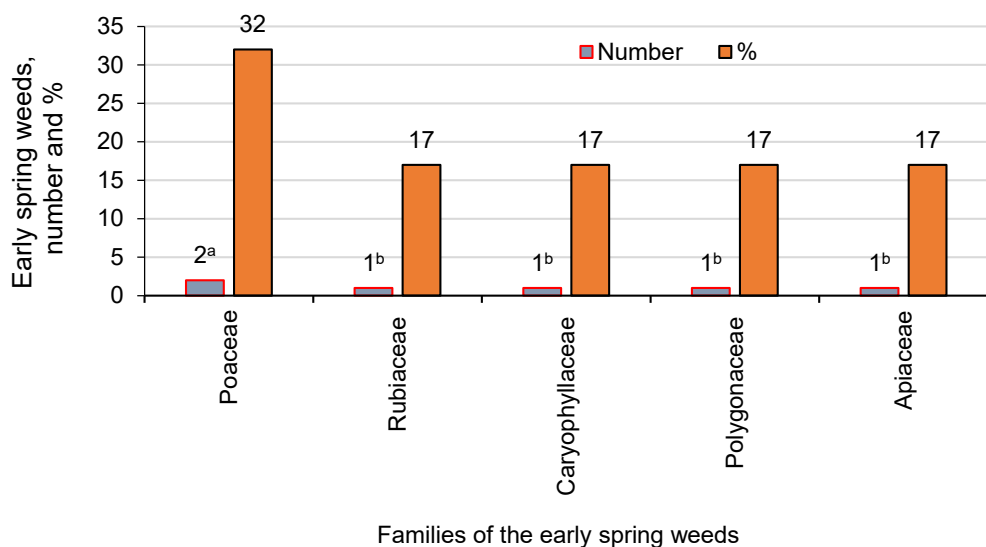


Fig. 5. Distribution of early spring weed species across botanical families.

Note: Means with different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

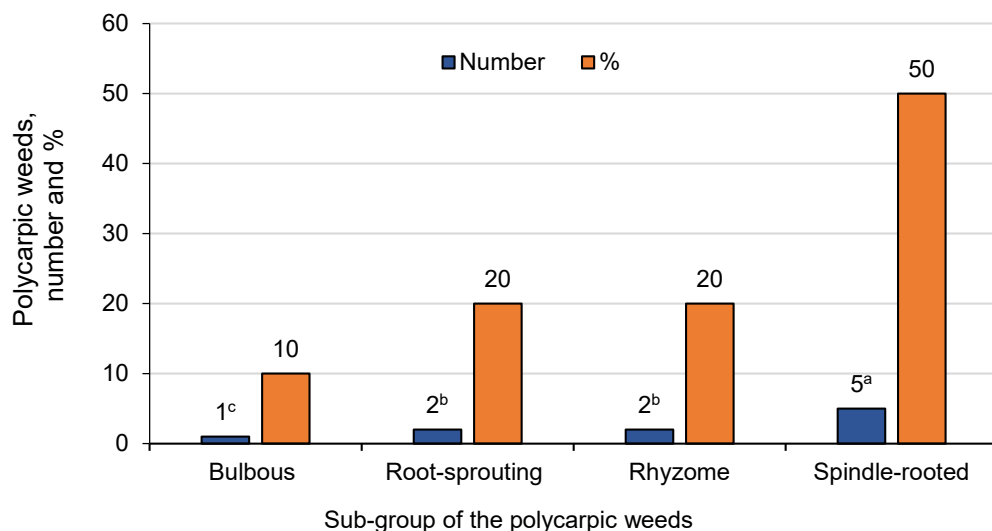


Fig. 6. Distribution of polycarpic (perennial) weed species by morphological subgroup.

Note: Means with different letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$).

grass (*Sorghum halepense*) and Bermuda grass (*Cynodon dactylon*).

Root-sprouting species were represented by the two most widespread species across most of the country's cultivated areas: field bindweed (*Convolvulus arvensis*) from the Convolvulaceae family and corn thistle (*Cirsium arvense*) from the Asteraceae family.

Representatives of bulbous species occupy 10 % of the total infestation. The registered species was grape hyacinth (*Muscari* spp.).

Conclusions

After the weed monitoring survey in the three sub-mountainous areas (the town of Brezovo and the villages of Streltsi and Varben), a high level of weed infestation was found. A total of 54 weed species were recorded, belonging to 22 botanical families.

The infestation was dominated by monocarpic weed species, while polycarpic weeds occurred at low density.

The most widespread weed species belonged to the Poaceae family. Annual grass weeds were more prevalent in the weed associations than the perennial species.

These new scientific findings from the weed monitoring, which enrich the knowledge of the weed flora in the studied sub-mountainous regions, will help farmers achieve more effective weed management in rotation of oilseed rape, followed by winter wheat.

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Diversity of local apple genetic resources in the Central Balkan Mountain region of Bulgaria

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Thematic field: Agriculture in mountainous and semi-mountainous regions

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Abstract

In Bulgaria, the apple (*Malus × domestica* Borkh.) has a long history of cultivation, and there is a wide diversity of genetic resources in the country. Wild relatives of this fruit species can be found in forests or other habitats. The local genetic resources are valuable due to their good adaptation to the climate and soil conditions of the natural habitat. In addition, they often have a number of valuable characteristics, such as resistance to diseases and pests, which should be conserved and used as donors for breeding programmes. The aim of the current study was to investigate the diversity of seedlings and local genotypes mapped during expedition studies in the Troyan region, Central Balkan Mountains of Bulgaria, in order to assess their characteristics and select the most valuable ones for future breeding purposes. For these purposes, the fruit size, flesh firmness, the total soluble solids content (°Brix), the skin and flesh colour were measured. All the characteristics studied were highly variable in the population. Fruit flesh colouration varied from white to pinkish. Regarding chemical analysis, very high sugar content was recorded in PM 11 (18.08 %), PM 12 (18.32 %), and PM 25 (17.44 %). All analysed samples had higher invert sugar content and lower sucrose content. Of all the studied genotypes, PM 17 and PM 104 had very low acidity. The studied local forms do not comply with modern market demands but are valuable for preserving biodiversity and for breeding purposes because of their good adaptation to the climatic conditions in the region.

Key words: biodiversity, local gene pool, local genotypes, *Malus × domestica*.

Introduction

The apple (*Malus × domestica* Borkh.) is one of the most widely distributed perennial fruit crops (Gross et al. 2014). This species is relatively old, domesticated initially at least 4000 years ago. Its wild relatives, distributed and grown in diverse habitats, are potentially useful sources of diversity for apple cultivation and breeding (Cornille et al. 2014). Fruit growing developed significantly in the 17th century, and accelerated urbanisation led to a drastic decline in wild and local forms of fruit species growing in nature (Nesheva et al. 2019).

In recent years, interest in the conservation and research of the gene pool of cultivated and wild fruit species has increased (Minkov and Mihova 2022). Plant genetic resources are of fundamental importance for agricultural science, mainly for fruit growing. Germplasm collections serve as important reservoirs of genetic diversity for crop improvement. They contain a great diversity that is essential for the identification of valuable phenotypes that can be used for the development of improved cultivars (Bramel and Volk 2019). Local genotypes are still preserved in many small settlements, especially in mountain areas (Bozhkova 2013). They are well adapted to the climatic and soil conditions of their natural habitat (Vitanov 1977). In addition, some genotypes have unique biological characteristics that should be preserved and used as donors for breeding programmes. The conservation of genetic resources is of regional and global importance. The thorough study of the most valuable characteristics and their description underlines their great importance. In order to collect, study, and preserve old and local fruit and grapevine cultivars and genotypes, a series of

expeditions were conducted throughout Bulgaria under the framework of the KP-06-N46/3 project funded by the Bulgarian National Science Fund (BNSF) of the Bulgarian Ministry of Education and Science.

Collecting and evaluating local germplasm is essential for the improvement of cultivars (Mratinic and Fotric-Aksic 2012). The current study aimed to investigate the diversity of seedlings and local genotypes mapped during the expedition studies in the Troyan region in the Central Balkan Mountain region of Bulgaria, in order to assess their qualities and select the most valuable ones for future breeding purposes.

Materials and Methods

In 2021, expedition studies were carried out in Troyan, the Central Balkan Mountain region of Bulgaria, and surrounding areas (Apriltsi, Chiflik, Beli Osam and adjacent villages and neighbourhoods) to document and characterise local genetic resources of fruit species, with a focus on vegetative and reproductive traits of historically marked and newly identified varieties. Over the following two years, 2021 and 2022, fruit quality and diversity were investigated in a population of 50 selected genotypes, many of which persist as single trees aged 85–100+ years (Table 1). These genotypes, often found at altitudes up to 1000 m and preserved due to favourable terrain and microclimatic conditions, reflect the area's rich heritage and potential for conservation and breeding.

An average fruit sample was taken from each genotype, and the biometric data, petiole length, fruit height and fruit diameter were measured with a Mitutoyo Digimatic Absolute Calliper 150 mm. Fruit shape was determined according to the

Table 1. List of the genotypes evaluated.

Sample code	Origin of samples	Identification/ Local name	Sample code	Origin of samples	Identification/ Local name
PM 1	Apriltsi region	FSP-1*	PM 102	Chiflik region	ChF-2
PM 2	Apriltsi region	Perusha var.	PM 104	Chiflik region	ChF-4
PM 4	Apriltsi region	FSP-3	PM 105	Chiflik region	ChF-5
PM 6	Apriltsi region	FSP-5	PM 106	Chiflik region	ChF-6
PM 7	Apriltsi region	FSP-6	PM 108	Chiflik region	Yellow Bellflower form
PM 10	Apriltsi region	Tsiganka var.	PM 107	Chiflik region	ChF-7
PM 11	Apriltsi region	Balkanska Ayvaniya	PM 112	Chiflik region	Tsiganka form
PM 12	Apriltsi region	Tsiganka var.	PM 109	Chiflik region	Byala yabalka form
PM 13	Apriltsi region	Cheshka momina	PM 110	Chiflik region	Troyanka-1 form
PM 14	Apriltsi region	FSP-10	PM 111	Chiflik region	Shekerka-1 form
PM 15	Apriltsi region	FSP-11	PM 113	Chiflik region	ChF-8 P.H.
PM 16	Apriltsi region	Jonathan	PM 114	Chiflik region	Limonka-1 form
PM 17	Apriltsi region	Ayvaniya var.	PM 118	Chiflik region	ChF-9
PM 18	Apriltsi region	FSP-12	PM 119	Chiflik region	Shekerka-2 form
PM 19	Apriltsi region	Zimna Petrovka	PM 120	Chiflik region	Zimna zelena
PM 21	Apriltsi region	FSP-13	PM 121	Chiflik region	ChF-10 A.M.
PM 22	Apriltsi region	Troyanka var.	PM 124	Beli Osam region	Zlatista yabalka
PM 25	Apriltsi region	FSP-15	PM 129	Beli Osam region	Reinette du Canada
PM 26	Apriltsi region	FSP-17	PM 1001	Beli Osam region	Belurka-1 form
PM 27	Apriltsi region	Reinette du Canada	PM 1002	Beli Osam region	Belurka-2 form
PM 29	Apriltsi region	VMF-2	PM 1003	Beli Osam region	Shekerka var. form
PM 39	Apriltsi region	FTM-2	PM 1004	Beli Osam region	Koravka var.
PM 43	Apriltsi region	Tsiganka var.	PM 1005	Beli Osam region	Koravka var. form
PM 44	Apriltsi region	FM-1	PM 1006	Beli Osam region	Shekerka var. form
PM 36	Apriltsi region	-	PM 1007	Beli Osam region	Shekerka var. form
PM 101	Chiflik region	ChF-1			

Note: *The local name is given by the abbreviation of the area where the genotype was found.

ECPGR descriptor by calculating the ratio of fruit height to fruit width (Lateur et al. 2022). Fruit weight was measured with a digital scale (Kern, Germany). The apple fruits were subjected to colour evaluation to CIE $L^*a^*b^*$, including Hue angle (h) and Chroma (c), using a PCE-CSM 2 (PCE Instruments, UK) handheld colourimeter with an aperture of 8 mm. The measurements were done on the equatorial part on both sides of 5 fruits. The firmness of the fruit was measured using a PCE-PTR-200N (PCE Instruments, UK) digital penetrometer after peeling the skin. The total soluble solids content (TSS) was mea-

sured using a Kern ORF 85BM (Kern, Germany) digital refractometer. The chemical analyses of the fruit flesh included the determination of total sugars, invert sugars and sucrose according to the method of Schoorl-Regenbogen (1952). The acidity (TA) was determined titrimetrically, using a titrator and the active acidity (pH) – potentiometrically, by a pH meter.

The data were processed by the Excel Data Analysis tool pack and IBM SPSS Statistics 26 software. Hierarchical cluster analysis using between-groups linkage was used to group the studied genotypes.

Results and Discussion

The fruit characteristics of the different cultivars and genotypes were highly variable. Red colour, low acidity, larger fruits, and firmness seem to have been under selection during the domestication and improvement of apple (Ma et al. 2015, Migicovsky et al. 2021). Fruit size is genetically determined, but is influenced by many agrotechnical and environmental factors. All genotypes studied developed under the same agro-environmental conditions and a diversity of biometric fruit data was observed (Table 2).

The highest variation was observed in fruit weight. The lowest apple fruit weight was 48.8 g (PM 19), and the highest was 156 g (PM 29). However, the analysis of the mean values of all measured parameters indicates a wide range of diversity between the local genotypes and does not meet the requirements of the market. For example, the fruits of the cultivar 'Florina' sold in Romanian supermarkets have a diameter of 65.6 mm and an average weight of 173.33 g (Iordănescu et al. 2021). The lowest deviation was observed in the fruit petiole length, which varied from 6.48 mm (PM 12) to 18.36 mm (PM 18).

The dimensions of the fruit determine its shape. According to the ECPGR descriptor (2022), fruit shape is determined by the fruit height/fruit width ratio. According to this, five groups were formed. Ratios smaller than 0.75 determine the flat shape, 0.76–0.85 determine a slightly flat shape, 0.86–0.99 – intermediate, 1–1.1

– slightly elongated and >1 – elongated. Three different shapes were observed in the group of apple genotypes studied. The intermediate shape was observed with the highest frequency (25 %), while the elongated and slightly elongated shape of the fruit was not observed in the analysed population (Fig. 1).

Due to their high nutritional value, apples are among the most widely consumed fruits worldwide. Ceci et al. (2021), comparing the chemical composition of old forms and commonly grown (commercial) cultivars, noted that the old forms were distinguished by the content of polyphenolic compounds and higher antioxidant capacity. Native wild, old and local apple genotypes are an important source of phytochemical compounds and are characterised by higher contents of bioactive compounds, which can be used as genetic material for apple breeding programmes (Balta et al. 2022). The quality of apples depends on the interaction of genetic and environmental factors (Begić-Akagić et al. 2014). Size, sweetness, and crispness are still the key traits and quality characteristics of apples, which are most important to consumers (Carrillo-Rodriguez et al. 2013). Sugars and organic acids are essential components that determine taste qualities (Wu et al. 2007, Bordonaba and Terry 2010). Sugars not only determine the sweetness of the fruit, but also serve as a carbon source for the synthesis of other components such as pigments, amino acids, vitamins, and aromatic compounds (Qin et al. 2025). Wild

Table 2. Diversity of the biometric data.

Characteristic	Minimum	Maximum	Mean	Std. Deviation
Fruit weight, g	48.80	156.00	95.73	±28.85
Fruit height, mm	39.52	65.08	50.68	±5.43
Fruit diameter, mm	48.33	73.33	59.99	±6.15
Petiole length, mm	6.48	18.36	12.63	±2.68

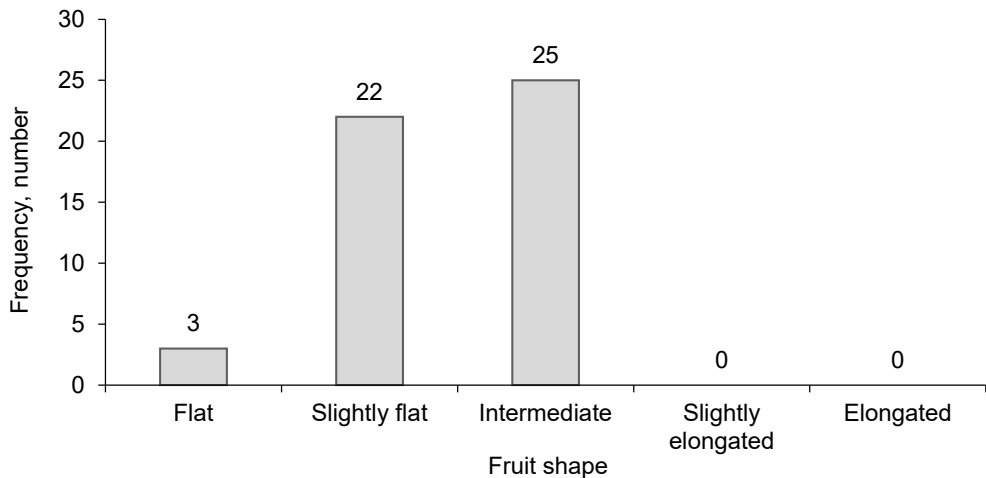


Fig. 1. Distribution of the accessions according to fruit shape.

and old local forms typically contain significantly higher contents of acids (malic acid) and a lower level of sucrose (Li et al. 2021) which corresponds well with our results, as most of the apple fruits studied exhibited a high total acidity together with high pH values. In the 50 apple samples examined, the TSS values ranged from 15.11 (PM 104) to 23.20 °Brix (PM 44) and are relatively high, given that TSS in commercial dessert apples generally falls between 12 and 18 °Brix (Bordonaba and Terry 2010) (Table 3). In the group studied, the average fruit firmness was 9.36 kgf·cm⁻². A substantial difference was observed in total sugar content, with values ranging from 6.88 % (PM 1001) to

14.90 % (PM 102). Invert sugars predominate in apple fruits. The sucrose content varied from 0.23 % (PM 1005) to 5.49 % (PM 107), which is 3 to 5 times lower than that of the invert sugar (from 4.50 % (PM 109) to 11.36 % (PM 1005)). Most of the apple fruits studied exhibited a high total acidity and high pH values. The deviation of both was low across the entire studied group.

The TSS content is an important fruit characteristic that is associated with the sugar content and the level of ripeness. It may vary within the same variety as a result of climatic factors (Gómez-Devia and Nevo 2024). There is a relationship between the TSS content and fruit firm-

Table 3. Diversity of the physical and chemical components.

Characteristic	Minimum	Maximum	Mean	Std. Deviation
TSS content, °Brix	15.11	23.20	19.11	1.96
Fruit firmness, kgf·cm ⁻²	4.90	12.81	9.36	1.78
Total sugars, %	6.88	14.90	10.50	2.20
Invert sugar, %	4.50	11.36	8.28	1.70
Sucrose, %	0.23	5.49	2.10	1.40
Acids (TA), %	0.26	1.31	0.76	0.22
pH	3.42	6.47	3.99	0.51

ness – the higher the TSS content, the riper and softer the fruits tend to be. Fruit firmness is strongly associated with consumers' preference for certain cultivars and is, therefore, a key target for breeders (Szczesniak 2002, Nybom et al. 2013). The firmness of the fruit flesh is relevant to an assessment of the fruit quality, the shelf life, and consumer acceptance (Hend et al. 2009). According to Harker et al. (2008) apple firmness is the dominant factor for consumer acceptance, but TSS and TA content also play a role in defining quality for specific cultivars. The largest were the groups of apple genotypes with a high TSS content. The group with TSS in the range of 17–19 °Brix and the group with TSS in the range of 19–21 °Brix, had a frequency of 32 % each (Fig. 2). A high percentage of the population also had a very high TSS over 21 °Brix. Sixty percent of the examined genotypes had an average fruit firmness in the range of 5–10 kgf·cm⁻² (Fig. 3).

A high deviation was found in the colour parameters describing the skin ground colour (Fig. 4). The hue angle was measured from around 20 to 105, showing the

variation from red to green skin ground colour. The L parameter reflects the variation in the brightness of the colouration. It is mainly between 40 and 80, indicating a lighter skin colouration. Genotypes with lower L values were identified in the studied population, indicating darker colouration. The green-red parameter a* was mostly around 0, suggesting a neutral balance between green and red tones. A higher a* value showed domination of red colouration, but it is not common in the studied population. Blue-yellow axis b* indicated a strong shift towards the yellow colouration of the skin ground colour. The chroma index (c*) measures the vividness of the skin colour and was around 50.

Less variation was observed in the fruit flesh colour parameters (Fig. 5). The hue angle of the population was around 90°, showing that the fruit flesh is in the yellow tones of the colour scale. One of the genotypes was interesting and different from the whole group, with a fruit flesh in the pink shade. In most of the studied genotypes, the colour of the fruit flesh is around 70°, suggesting that the flesh is quite light, nearly white or creamy in colour. The a*

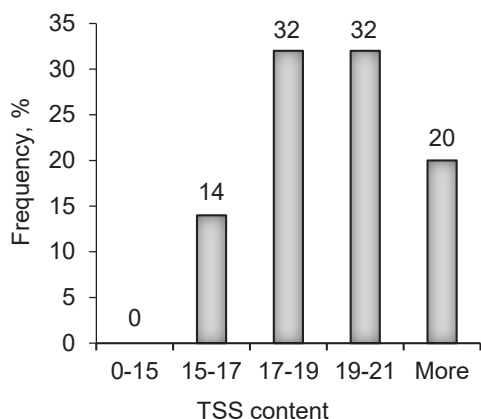


Fig. 2. Distribution of the accessions according to TSS content, °Brix

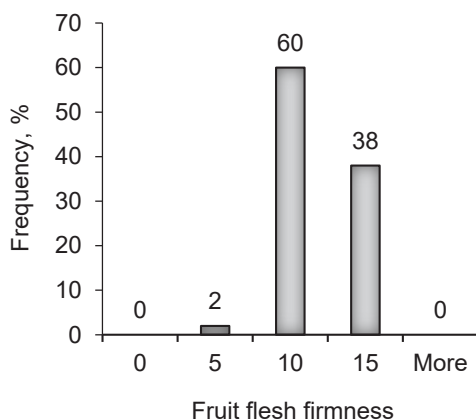


Fig. 3. Distribution of the accessions according to the fruit firmness, kgf·cm⁻²

Colour parameter ● L ● a ● b ● c ● h

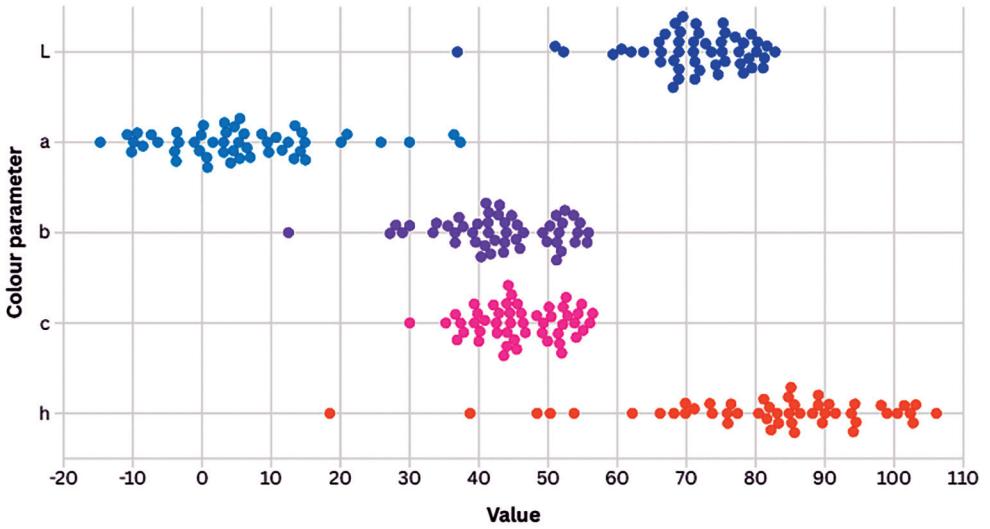


Fig. 4. Distribution of the colour parameters of the skin ground colour in the studied group.

Colour parameter ● L ● a ● b ● c ● h

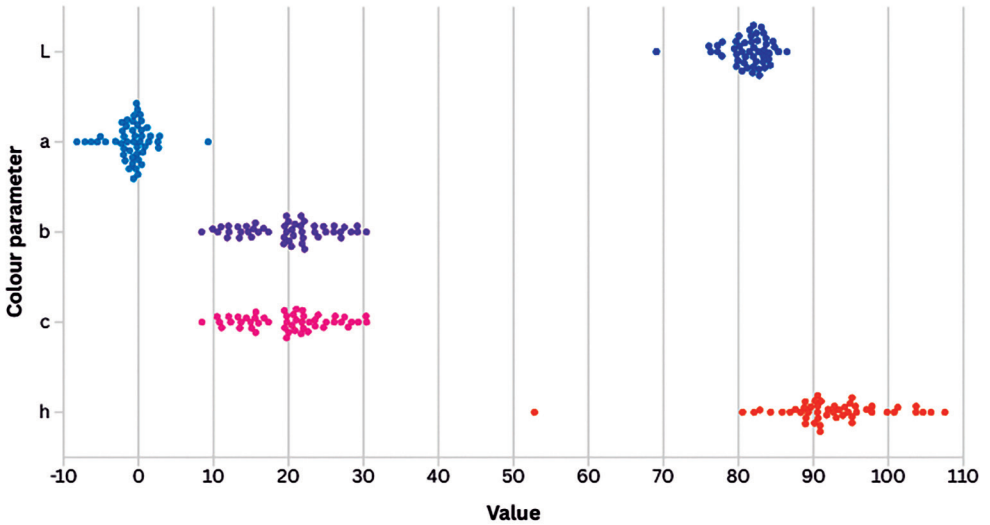


Fig. 5. Distribution of the colour parameters of the fruit flesh in the studied group.

value was tightly grouped around 0, which means the flesh does not lean distinctly green or red. The genotype with the pink

colouration of the flesh had the highest value of a^* . For most of the genotypes, the b^* parameter was around 20, indicating a

mild yellow colouration of the fruit flesh. Chroma index was also close to 20, so that yellow hue is present but not a highly saturated colour.

A cluster analysis was performed to group the studied accessions based on their similarities in the biometric and chemical characteristics. According to the biometric data, this statistical method revealed two main groups (Fig. 6). Cluster one includes accessions with very

small, small, and small to medium fruits (Lateur et al. 2022) and with longer petioles – from PM 15, with an average fruit weight of 86.7 g, FH – 49.76 mm, FW – 58.9 mm, and PL – 13.85 mm, to PM 19, with an average fruit weight of 48.8 g, FH – 43.77 mm, FW – 43.32 mm, and PL – 15.04 mm. Cluster two includes the accessions with larger fruits and short petioles, from PM 1 to PM 120, with sizes ranging from small to medium, medium, or medi-

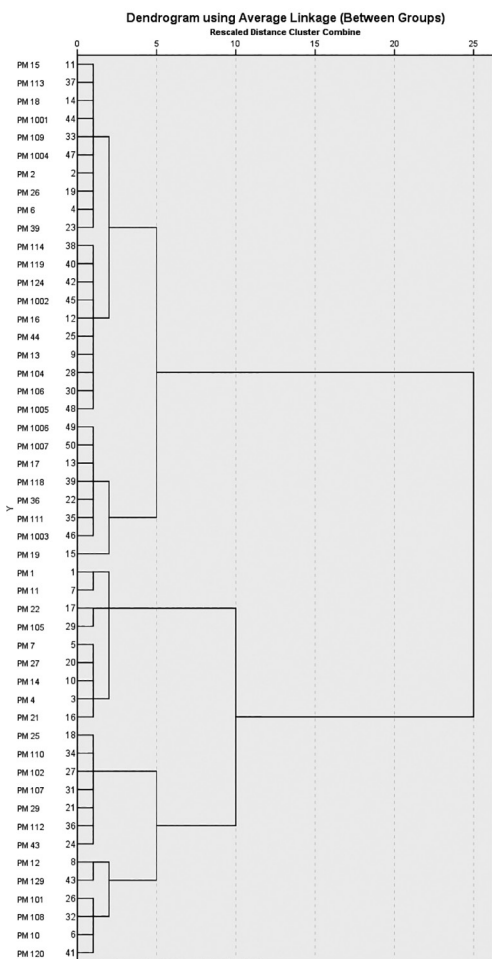


Fig. 6. Dendrogram of grouping of the genetic resources by their biometrical data.

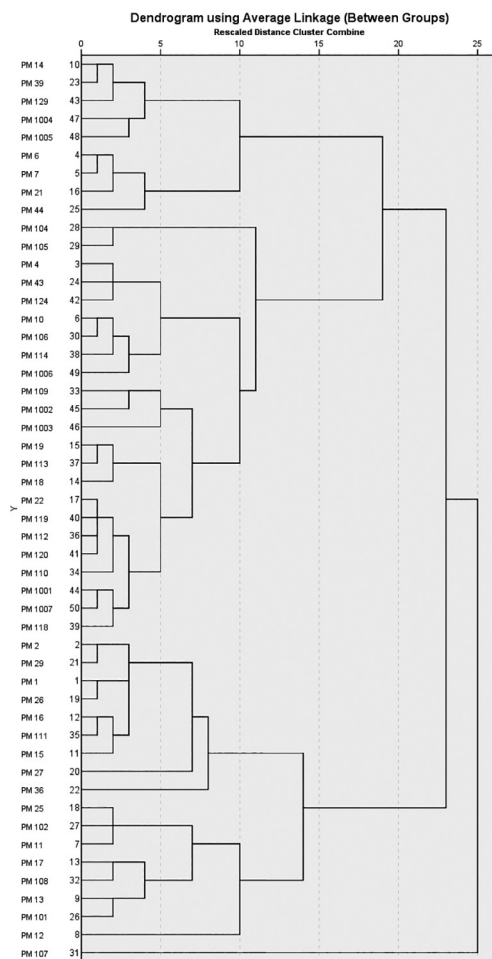


Fig. 7. Dendrogram of grouping the genetic resources by their chemical components.

um to large. In the last group with medium to large fruits, only PM 12 (fruit weight 156 g, FH – 52.51 mm, FW – 73.33 mm, PL – 6.48 mm) and PM 129 (fruit weight 151.4 g, FH – 56.82 mm, FW – 71.29 mm, PL – 10.12 mm) are included. The genotypes in the second cluster comprise 44 % of the studied group and are more valuable due to their greater fruit size.

The apple genotypes analysed could also be divided into 2 main groups according to the chemical components. Forty-nine accessions form one large group with similar chemical content. PM 107 was identified as the most different from the others (Fig. 7). This accession formed a separate cluster and was the only one with a high sucrose content compared to the others (5.49 %). The remaining 49 apple genotypes form four different clusters. Cluster 2, formed from PM 14 to PM 44, includes 9 genotypes with very high TSS content (above 20 °Brix), high invert sugars and low sucrose content. Cluster 3, formed by PM 104 to PM 118, combines genotypes with average values of the examined chemical characteristics. Cluster 4 – PM 2 to PM 36 – is represented by 9 accessions with low total acidity levels (from 0.49 % for PM 111 to 1.02 % for PM 27). Cluster 5 contains PM 25 to PM 12, which have a high total sugars content from 11.95 % for PM 101 to 14.89 % for PM 102, high invert sugars from 7.72 % (PM 17) to 11.04 % (PM 11), high sucrose from 3.08 % (PM 101) to 5.12 % (PM 12) and low total acidity from 0.25 % (PM 17) to 1 % (PM 12).

Conclusions

The fruit quality of local genetic apple resources was investigated using standard methodologies and techniques. The ge-

netic resources analysed are not oriented towards today's quality standards for apple fruit. However, a rich diversity of different and interesting, old and local genotypes is a prerequisite for the inclusion of new genitors in breeding programmes.

The genotypes with larger fruits and higher sugar content, such as PM 12 and PM 129, are particularly valuable for breeding programmes aimed at improving fruit quality. A natural genotype (PM 18) with pink fruit flesh was found, which can be used as a donor to introduce desirable traits into new cultivars.

The local apple genotypes are well adapted to the climatic and soil conditions of the Central Balkan region. This adaptation makes them resilient to local environmental stresses and valuable for the development of cultivars that can thrive under similar conditions.

The study underlines the importance of ongoing research and development efforts to utilise the genetic resources of local genotypes. Continued research and characterisation of these resources will support the development of breeding programmes.

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