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Pan-European Criteria for Sustainable Forest Management - Attitudes of Forestry Professionals in the Federation of Bosnia and Herzegovina

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ABSTRACT

Climate change is recognized as a global threat that negatively impacts biodiversity and forest resources. The use of existing indicators for sustainable forest management (SFM) related to biodiversity and climate change, as well as the development of new indicators, will help assess how forest management practices impact biodiversity enhancement and climate change mitigation. A Pan-European set of criteria and indicators has been developed as a policy instrument for monitoring, evaluating, and reporting on the progress in implementing SFM. In Bosnia and Herzegovina and Western Balkans in general, the Pan-European set of criteria and indicators is an insufficiently researched topic and there is a lack of scientific research conducted regarding their development and implementation. Through the analysis of the current situation in forestry of the Federation of Bosnia and Herzegovina (FBiH), regarding the compliance and importance of the Pan-European criteria for SFM, this paper aims to explain how the international process of development and application of the Pan-European criteria for SFM can contribute to the improvement of the situation in forestry and the creation of a consistent forest policy in FBiH. The survey among forestry professionals (n=360), from the public forest administration and public forest companies in FBiH, included the sets of questions related to socio-demographic characteristics, assessment of compliance and importance of six criteria of SFM. Research results revealed that forestry professionals are mainly males, on average 41 years old, with 13 years of working experience. The majority of forestry professionals in FBiH are not familiar with Pan-European criteria for SFM, and have a low level of their understanding. On average, forestry professionals indicated that the Pan-European criteria for SFM were of high importance, while compliance with current forest management activities were rated lower on average. The large differences between responses regarding the average rating of compliance and importance of the Pan-European criteria for SFM indicate their low level of implementation in FBiH forest management activities. Accordingly, the results indicate that there is a need to organize educational lifelong learning programs in FBiH forestry sector, involving forestry professionals and other interested parties, to generate knowledge related to the Pan-European criteria for SFM and the concept of SFM in general.

Keywords: Federation of Bosnia and Herzegovina; sustainable forest management; sustainable forest management criteria and indicators; forestry professionals; compliance; importance

INTRODUCTION

Nowadays, the concept of sustainability is being integrated into forestry activities. Due to the current socio-economic realities, the concept of sustainable management of forest resources is recognised, among other things, as a process of meeting the present needs of society through

the sustainable use of forest resources, together with the continuous improvement of forest conditions, which should also meet the needs of future generations (Sample and Sedjo 1996). It is important to note that people are at the centre of the concept of sustainable forest resource management, with the aim of meeting the various needs of present and future generations (FAO 2022). Various sectoral

policies (climate policy, energy policy, nature conservation policy, etc.) place complex and changing demands on forest resources at the global level, while forest science tries to find solutions that satisfy the interests of various forest-related stakeholders by applying the concept of sustainable participatory management and a multifunctional approach. Following the United Nations Conference on Environment and Development (Rio de Janeiro 1992), international processes and initiatives were launched to formulate criteria and indicators for sustainable forest management (SFM). The criteria and indicators are intended to serve as a forest policy instrument for monitoring, evaluating and reporting on the application and progress of the concept of SFM at global, regional and national levels (Barbati et al. 2013, EFI 2013). In Europe, the Ministerial Conference on the Protection of Forests (formerly MCPFE, now Forest Europe) led the way in defining a set of criteria and indicators for SFM and applying them in practise. The criteria and indicators for SFM have been further developed and adapted over time, while their application has led to the production of national reports on SFM, summarised by Forest Europe in the State of European Forests Report (Forest Europe 2020). Further development of SFM criteria and indicators is foreseen in the areas of ecosystem health, biodiversity and climate change, so that they can become a more detailed screening tool for assessing SFM (European Commission 2021).

With regard to the social, ecological and economic dimensions of forestry, the concept of SFM has historically evolved and become an indispensable paradigm of modern forestry (Floyd 2002). However, in order to assess progress in achieving SFM, it has been necessary to define and develop criteria and indicators for SFM (Wijewardana 2008, Pölzl and Rametsteiner 2009, Rametsteiner et al. 2011). The Pan-European set of criteria and indicators has been researched through several international projects (EFI 2013, FAO 2016, UNECE 2017). In the last few decades, the criteria and indicators of SFM have been more intensively researched, whether it is the implementation of Pan-European criteria and indicators of SFM at the international (Baycheva-Merger and Wolfslehner 2016), regional, national or subnational level (Duinker 2001, Hickey and Innes 2005, Santopuoli et al. 2016, Linser et al. 2018a, 2018b, Linser and Wolfslehner 2022).

When it comes to the Western Balkans region and Bosnia and Herzegovina (BiH), the Pan-European criteria and indicators for SFM are an insufficiently researched topic and there is no significant scientific research on their implementation in forestry. Some authors studied the contribution of SFM based on scientific postulates of sustainable development in BiH (Avdibegović et al. 2022). One of the few papers published in the Western Balkans region that addressed the issue of SFM criteria and indicators focused on assessing progress in SFM in Croatia through the use of quantitative, improved Pan-European criteria and indicators (Lovrić et al. 2010). In BiH, an overview of the set of generic Pan-European criteria and indicators from 2003 was published (Lojo 2016). In addition, authors addressed the possibilities of applying FSC principles and criteria as external standards in the certification process (Avdibegović 2001, 2002, 2004) and examined the set of principles of the concept of "forest governance" in FBIH forestry (Mutabdzija 2012, Avdibegović et al. 2014a, 2017), as well as the

analysis of corrective actions in obtaining and maintaining the FSC certificate (Avdibegović et al. 2014b, Halalisan et al. 2016, Pezdevšek Malovrh et al. 2019). Despite the existing research, there is an apparent lack of scientific work focusing exclusively on the issue of Pan-European criteria and indicators of SFM in BiH from a forest policy perspective.

Due to BiH's unique constitutional framework, there is neither a long-term strategy for forest development, nor a coherent forest policy, nor a forest law framework at the state level. Forest policy is decentralised and shaped by the entities (Republic of Srpska and FBIH) and the Brčko District. Institutions at these levels are responsible for shaping forest policy as well as forest legislation and its implementation (FAO 2015). Therefore, there is a need to analyse the possibilities of applying the Pan-European criteria for SFM in FBIH forestry. In this sense, this research represents a pioneering work based on empirical research in which the level of importance of and compliance with six criteria for SFM was analysed. In that sense, this paper aims to explain how the international process of development and application of the Pan-European criteria for SFM can contribute to the improvement of the situation in forestry and the creation of a consistent forest policy in FBIH.

MATERIALS AND METHODS

The Pan-European set of criteria and indicators for SFM consists of 6 criteria, 34 quantitative and 11 qualitative indicators (Forest Europe 2015). The Pan-European criteria and indicators for SFM are basic instruments for defining, applying and promoting SFM by providing relevant information for the development and evaluation of national forest policies, plans and programmes. They serve as a basis for collecting cross-sectoral data on forest management and as a means of communicating the forest sector to the general public. With their help, it is possible to monitor, evaluate and report on progress in SFM at regional and national levels (MCPFE 1998). In this sense, this paper analyses the level of familiarity of forestry professionals in FBIH (employees of public forest companies and institutions of public forest administration) in relation to the Pan-European criteria for SFM. In addition, the paper addresses the assessment of the level of compliance and importance of six Pan-European criteria for current forestry activities in FBIH (Table 1). It becomes clear that the paper focuses on three different aspects of the assessment:

1. Familiarity – indicates the level to which respondents are familiar with the Pan-European set of criteria for SFM;
2. Compliance – indicates the level of compliance of the Pan-European set of criteria for SFM with current forest management activities in FBIH;
3. Importance – indicates the level of importance of the Pan-European set of criteria for SFM for current forest management activities in FBIH.

A survey of university graduated forestry professionals was conducted in FBIH to determine their level of familiarity, level of compliance and level of importance regarding the set of six Pan-European criteria for SFM (Table 1). In 2018, public forest companies and institutions of public

Table 1. Pan-European criteria for sustainable forest management (Forest Europe 2020).

Criterion 1	Maintenance and appropriate enhancement of forest resources and their contribution to global carbon cycles
Criterion 2	Maintenance of forest ecosystem health and vitality
Criterion 3	Maintenance and encouragement of productive functions of forests
Criterion 4	Maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems
Criterion 5	Maintenance and appropriate enhancement of protective functions in forest management
Criterion 6	Maintenance of other socio-economic functions and conditions

forest administrations (Ministry of Agriculture, Water Management and Forestry of FBiH, Forestry Office of FBiH, Cantonal Forestry Offices) were contacted in order to obtain information on the number of employed university graduated forestry professionals in FBiH (N=538). Since the potential respondents are employees of the public institutions who are relatively easy to reach, it was decided to distribute the questionnaires to all identified university graduated forestry professionals, so that the sample size corresponded to the population size. The questionnaires were delivered in person to forest companies and public forest administration institutions. A total of 538 questionnaires were distributed and 360 completed questionnaires were returned (313 from public forest companies and 47 from public forest administration), representing response rate of almost 67%. The questionnaire was designed to provide a quantitative description of the trends, attitudes or opinions of the respondents in the sample, which allowed generalisation of the conclusions about the studied population (De Vaus 2002). The questionnaire consisted of five sections asking for information on socio-demographic characteristics of university graduated forestry professionals in FBiH (gender, age, education, number of years of work experience, type of institution where the respondent is employed), familiarity with the Pan-European criteria of SFM, assessment of the level of compliance and the level of importance of each of the six criteria of SFM by using the five-point Likert scale. The explanation of the set of Pan-European criteria for SFM with corresponding indicators was provided prior to the part where the respondents gave their assessment grade for the level of compliance and level of importance. The fourth section contained questions on the possible use of Pan-European criteria and indicators for SFM, on the responsible institutions for the implementation of Pan-European criteria

and indicators for SFM and on the responsible institution for data collection. The fifth section was dedicated to identifying possible difficulties in implementing the Pan-European criteria and indicators for SFM in FBiH. The questionnaire was tested among staff of public forest companies, institutions of public forest administration and staff of the Faculty of Forestry of the University of Sarajevo.

The collected data were entered into MS Excel 2016 and processed in the statistical analysis programme SPSS 22. The statistical analyses in this study were conducted in two stages. In the first stage, all the collected data were presented by using the frequency distribution, i.e., the selected location measures (mean, maximum and minimum). In the second stage, the Mann-Whitney U-test was used to test the differences between the respondents' assessments of the level of compliance and importance of Pan-European criteria for SFM for forest management activities in FBiH, according to gender, age, work experience, education and type of institution. Some of the variables used in these tests were recoded (Table 2). As the criteria of normal distribution and homogeneity of variance of the data were not met, non-parametric tests were used (Field 2009). Statistically significant differences were defined as $p < 0.05$.

RESULTS AND DISCUSSION

Socio-Demographic Characteristics of Respondents

The socio-demographic characteristics of the respondents are shown in Table 3. It can be seen that the majority of the respondents are male, 77.8%. Despite the positive trend in the number of female students in forestry (FAO 2020), more men than women traditionally enrol in forestry faculties. The results of the latest survey

Table 2. Independent variables for the "Mann-Whitney U" non-parametric test.

Independent variables	Coded variables
Gender	1 - Male 0 - Female
Age	1 – up to 40 0 – more than 40
Work experience in forestry	1 – up to 10 0 – more than 10
Education	1 – Bologna system of education 0 – Pre-Bologna system of education
Type of institution	1 – Public forest company 0 – Public forest administration

show that most respondents perceive the forestry sector as a male domain and that the vast majority believe that the representation of women is not satisfactory (Forests in Women's Hands 2021).

As for the average age of the respondents, the results show that the respondents are on average 41 years old, while the youngest respondent was 22 years old and the oldest 65 years old. In terms of the age structure of employees, young employees are most represented in the age group between 20 and 40 years (57.5%), 12.1% of the respondents are middle-aged, while 30.40% of the respondents are in the age group between 51 and 65 years. The results obtained in this study are similar to the results of another study (e.g. Mutabdžija 2012).

Results also indicate that the largest group in the sample are forest engineers with a four-year university education (67.8%). The second largest group in terms of representation are Masters of forestry, making one-fifth of the sample (19.7%). Third are Bachelors of forestry making 6.9%, fourth is the group of respondents who belong to Masters of forestry sciences making 5%, and the smallest group were PhDs in forestry sciences making 0.6% of sample. In the future, a decrease in the number of engineers in the total number of forestry professionals in FBiH can be expected due to their retirement and the fact that the four-year forestry degree programme (pre-Bologna system of education) is no longer formally active in FBiH territory. This research has shown that the number of employed bachelor graduates in public forest companies is increasing, compared to the previous research (Mutabdžija 2012), and that 25 bachelor graduates have been hired. In addition, the majority of university graduated forestry professionals (86.9%) are employed in FBiH in public forest companies in FBiH, while 13.1% of the respondents are employees of

public forest administration institutions.

On average, forestry professionals in FBiH have 13.7 years of work experience (1 year minimum and 38 years maximum). The data on the age structure and work experience of the respondents can make an important contribution to the planning of the educational policy of higher education institutions in the field of forestry in BiH. A high percentage of younger population among forest engineers/masters might indicate the need to reduce the number of vacancies for enrolment of forestry students in the near future. The decision on the number of enrolled students should be taken after a comprehensive analysis of all possible and available jobs in the FBiH forestry sector. Another step towards understanding and using this data should be in the direction of diversifying and expanding the teaching programmes at the Faculty of Forestry, as it is logical that forestry professionals with university diplomas will be forced to seek employment outside the forestry sector in the near future (Mutabdžija 2012).

Familiarity with the Pan-European Criteria for SFM

The average rate of familiarity with the Pan-European criteria for SFM among the respondents from the sample is 1.92. About one-third of the respondents (33.1%) are not at all familiar with the Pan-European criteria for SFM. Less than half of the respondents (43.7%) are slightly familiar, around one-fifth of the respondents (21.3%) are somewhat familiar, while only 2.0% of the respondents are very familiar with the Pan-European criteria for SFM (Figure 1).

Figure 2 shows the respondents' answers on familiarity with the Pan-European criteria for SFM by type of institution. It shows that the respondents' familiarity with the Pan-European criteria for SFM is slightly higher among respondents employed in public forest administration (30%) than among respondents employed in public forest

Table 3. Socio-demographic characteristics of the respondents (n=360).

Variable	Category	Percentage of respondents
Gender	Male	77.8
	Female	22.2
Age	20-30 years	21.7
	31-40 years	35.8
	41-50 years	12.1
	51-60 years	27.9
	More than 60 years	2.5
Education	Bachelor	6.9
	Graduated engineer	67.8
	Master of forestry	19.7
	Master of forest sciences	5
	PhD	0.6
Work experience	Up to 10 years	47.6
	11-20 years	25.2
	21-30 years	19.9
	More than 30 years	7.3
Type of institution	Public forest company	86.9
	Public institutions of forest administration	13.1

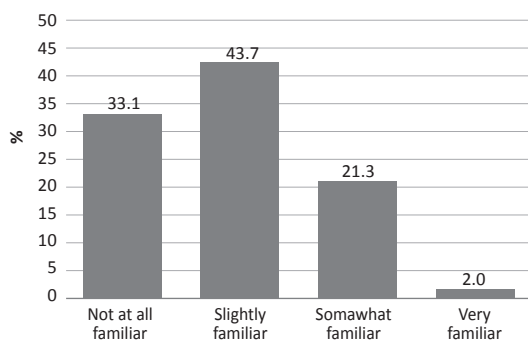


Figure 1. Familiarity with the Pan-European criteria for SFM.

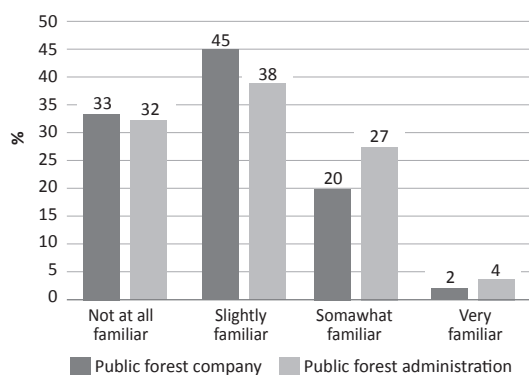


Figure 2. Familiarity with Pan-European criteria for SFM by the type of institution.

companies. However, the results of the Mann-Whitney U-test show that these differences between respondents from the different types of institutions are not statistically significant ($p=0.709$). This indicates that the majority of respondents are not at all familiar or only slightly familiar with the Pan-European criteria for SFM and that the type of institution they belong to has no influence on the level of familiarity. In view of this, it can be concluded that there is a need for lifelong learning and continuous improvement of existing knowledge about the Pan-European criteria and indicators for SFM.

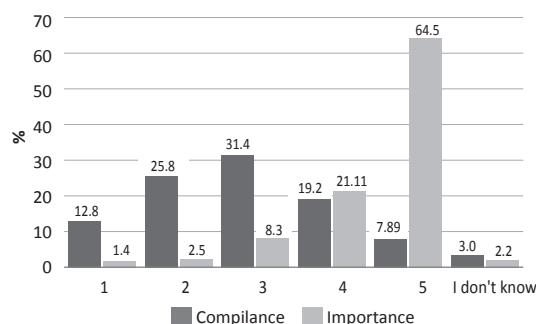
Assessment of Compliance and Importance of Pan-European Criteria for SFM with the Forest Management Activities in FBiH

The level of compliance and the level of importance of six Pan-European criteria for SFM were investigated using a Likert scale in the questionnaire. Figure 3 shows the distribution of answers related to the level of compliance and level of importance for Criterion 1 "Maintenance and appropriate enhancement of forest resources and their contribution to global carbon cycles with forest management practices". This criterion is defined by analysing indicators/information on forest area, growing stock, age structure and/or diameter distribution and forest

carbon, as well as policies, institutions and instruments to maintain and appropriately enhance forest resources and their contribution to global carbon cycles. The compliance of the Criterion 1 with the forest management activities in FBiH are considered poor and very poor (38.6%) or neutral (31.4%) by the majority of the respondents with the average ratings of 2.54. Further statistical analysis regarding the level of compliance, presented in Table A1 (see Appendix), related to the differences between ratings of compliance according to gender, age, working experience, education and type of institutions (Mann-Whitney U-test), shows that there are no statistically significant differences in the respondents' ratings. When it comes to the level of importance of Criterion 1, the majority of the respondents (over 85%) rate it as important or very important for forest management activities in the FBiH indicated by the average rating of 4.36. The results of Mann-Whitney U-test (Table A2 in the Appendix) for the level of importance showed that the gender ($p=0.004$) and educational level ($p=0.035$) have statistically significant influence on average ratings. In general, female respondents and respondents with diploma of pre-Bologna system of education, on average, gave higher rates of importance than male respondents and respondents with diploma of Bologna system of education.

It can be seen that there is a difference in the respondents' answers between the level of compliance and the level of importance for the Criterion 1. While the Criterion 1 is considered as very important in general, the respondents perceived the compliance level mostly as very poor. This implies the need for education on specific indicators related, among others, to the circulation and storage of carbon within the Criterion 1.

Some of the information required for the assessment of Criterion 1 can be found in various international and national information sources. In the latest Forest Europe reports for BiH (2020), the percentage of total forest area has remained quite stable and without major changes, and now stands at 54.9% of the total area, including a variety



Compliance	1 Very poor	2 Poor	3 Neutral	4 Good	5 Very Good
Importance	1 Not important	2 Slightly important	3 Neutral	4 Important	5 Very important

Figure 3. Assessment of compliance and importance of Criterion 1 "Maintenance and appropriate enhancement of forest resources and their contribution to global carbon cycles with forest management practices".

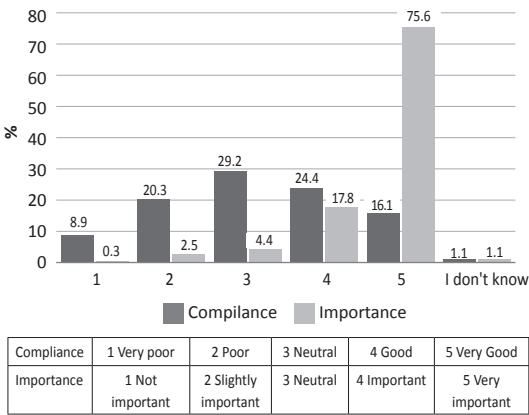


Figure 4. Assessment of compliance and importance of Criterion 2 "Maintenance of forest ecosystem health and vitality".

of land uses considered as other wooded land. However, the wood stock has steadily increased, reaching 230 m³ o.b. per ha in the public forests. The same data for soil biomass has been continuously reported since 2000 without any changes, so the trend cannot be assessed. Furthermore, no information was provided on the carbon stock in harvested wood products (Forest Europe 2020). The official data in the ministerial document Information on Forest Management in FBiH for 2021 and the Forest Management Plans for 2022 contain data on forest area, timber stock, age structure and/or diameter distribution, but no data on forest carbon stock (FMAWF 2022).

Figure 4 shows the distribution of answers related to the assessment of the level of compliance and importance of Criterion 2 "Maintenance of forest ecosystem health and vitality". This criterion is defined by analysing indicators/information on the deposition and concentration of air pollutants, soil condition, defoliation, forest damage, forest land degradation and policies, institutions and instruments to maintain forest ecosystem health and vitality of forest ecosystems. Almost 30% of the respondents had a neutral attitude regarding the compliance of Criterion 2, while over 40% of the respondents believe that Criterion 2 is good and very good in compliance with forest management practices, which is indicated with relatively high average ratings of 2.95. Similar to Criterion 1, further statistical analysis of the differences between ratings of compliance according to the independent variables shows that there are no statistically significant differences in the respondents' ratings - Table A3 (see Appendix).

On the other hand, the majority of the respondents (93.4%) believe that Criterion 2 is important and very important for forest management activities in FBiH, with the average ratings of 4.58 (Figure 4). The results of Mann-Whitney U-test (Table A4 in the Appendix) showed that the female respondents ($p=0.006$), the older ($p=0.009$) and more experienced respondents ($p=0.001$), those with diploma of pre-Bologna system of education ($p=0.002$) and respondents employed in public forest companies ($p=0.0029$) gave the higher average ratings to the level of importance for Criterion

2. Despite the relevance of Criterion 2, official statistics for the forestry in FBiH provide limited data on these concerns, which are mostly related to forest damage caused by forest fires or illegal logging (FMAWF 2022). Existing information needs to be improved by integrating data on deposition and concentration of air pollutants, soil condition, defoliation and degradation of forest areas, and compiling them into a single comprehensive database at FBiH level.

Figure 5 shows distribution of answers of the respondents' attitudes related to the assessment of the level of compliance and importance of Criterion 3 "Maintenance and encouragement of productive functions of forests". This criterion is defined by analysing indicators/information on increment and felling, roundwood, non-wood goods and services, as well as policies, institutions and instruments to maintain and encourage the productive functions of forests. The results show that the majority of the respondents (46.9%) believe that Criterion 3 is good and very good in compliance with forest management practices in FBiH, while 30.5% of the respondents have a neutral attitude on this issue. This attitude is supported by the average grade of the compliance of 3.13. As with the previous criteria, statistical analysis of the differences between compliance ratings as a function of the independent variables (gender, age, work experience, education and type of institution) shows that there are no statistically significant differences in the respondents' ratings - Table A5 (see Appendix).

Regarding the assessment of the importance of Criterion 3, the majority of the respondents (93.3%) consider that this criterion is important and very important for forest management activities in FBiH (average rating 4.58). The results of the Mann-Whitney U-test (Table A6 in the Appendix) for the level of importance show that gender ($p=0.017$) and level of education ($p=0.047$) have a statistically significant influence on the average ratings. Similar to Criterion 1 and Criterion 2, female respondents and those with a degree from the pre-Bologna system rate the importance of Criterion 3 higher on average than male respondents and those with a degree from the Bologna system. The importance of the productive functions of forests is reflected in the fact that many

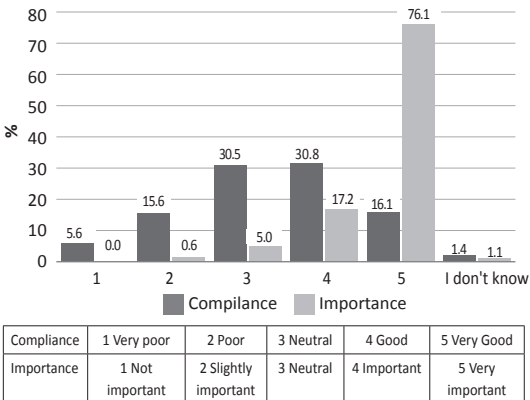


Figure 5. Assessment of the compliance and importance of Criterion 3 "Maintenance and encouragement of productive functions of forests".

local communities depend directly or indirectly on forests and forest products and services. The continuous and sustainable provision of forest services is linked to their productive capacities, which must not be exceeded, otherwise ecosystem disruption could occur. In this sense, it is necessary to plan and implement activities that ensure the sustainable use of forest products and services in a way that does not impair the functioning of forest ecosystems. In order to meet society's needs for forest products (timber and non-timber) and services (ecosystem service bundles), it is necessary to follow trends in social, economic and technological development, as these are directly related to society's changing demands on forests. Forest management activities must be carefully balanced with the aim of meeting the needs of current generations without destroying or compromising the ability of future generations to use the productive functions of the forest, while respecting the traditional needs and uses of forests by local communities and populations (Avdibegović et al. 2022). Although the vast majority of the respondents consider this criterion important, official data only include statistics on increment, annual allowable harvest and logging, but no information on non-timber goods and services (FMAWF 2022).

Figure 6 shows the results of the respondents' attitudes towards assessing compliance of Criterion 4 "Maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems" with the forest management activities in FBiH. This criterion is defined through analysis of the indicators/information on tree species diversity, regeneration, naturalness, introduced tree species, deadwood, genetic resources, forest fragmentation, threatened forest species, protected forests, common forest bird species and policies, institutions and instruments for conservation, and preservation and adequate enhancement of biodiversity in forest ecosystem. The average rating for this criterion is 2.92 and more than 39% of the respondents indicated that Criterion 4 is good and very good in terms of compliance with forest management activities in FBiH. Statistical analysis did not reveal any statistically significant differences in respondents' ratings in terms of differences in compliance according to the selected variables - Table A7 (see Appendix).

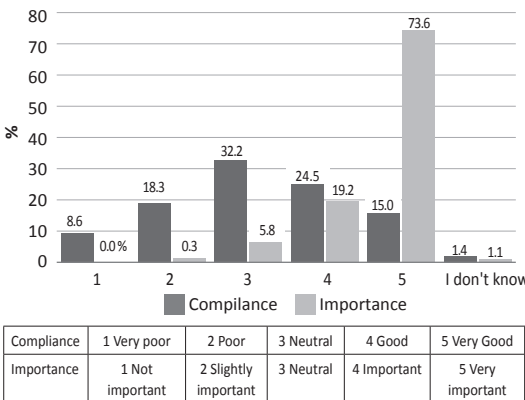


Figure 6. Assessment of the compliance and importance of Criterion 4 "Maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems".

The assessment of the importance of Criterion 4 for forest management activities in FBiH (Figure 4) showed that 92.3% of the respondents rated this criterion as important or very important, with an average rating of 4.54. The results of the Mann-Whitney U-test (Table A8 in the Appendix) for the level of importance showed that gender ($p=0.001$) and age ($p=0.020$) had a statistically significant influence on the average ratings. On average, female respondents and respondents older than 40 years gave higher ratings of importance of Criterion 4 for forest management activities in FBiH. Currently, information on indicators for tree species diversity, regeneration, naturalness, introduced tree species, deadwood, genetic resources, threatened forest species, protected forests and common forest bird species is not included in the official data (FMAWF 2022). According to the latest Forest Europe report, 4.0% of forest and other wooded land is protected for biodiversity conservation (MCPFE classes 1 and 2) (Forest Europe 2020). Data on threatened forest species are available in the Red List of Threatened Wild Species and Subspecies of Plants in FBiH (Greenway 2013).

Figure 7 shows the distribution of the respondents' answers in relation to the assessment of compliance with Criterion 5 "Maintenance and appropriate enhancement of protective functions in forest management". The definition of this criterion is based on the analysis of indicators/information on protective forests (protection of soil, water and other ecosystem functions - infrastructure and managed natural resources to prevent natural disasters) and policies, institutions and instruments to maintain and adequately improve protective functions in forest management. In the survey conducted, 49.2% of the respondents evaluated Criterion 5 as good and very good in terms of compliance with forest management practises in FBiH, while 26.7% of respondents had a neutral attitude towards this criterion, with an average rating score of 3.09. Further statistical analysis of the differences between ratings of compliance according to the independent variables, shows that there are no statistically significant differences in the respondents' ratings - Table A9 (see Appendix).

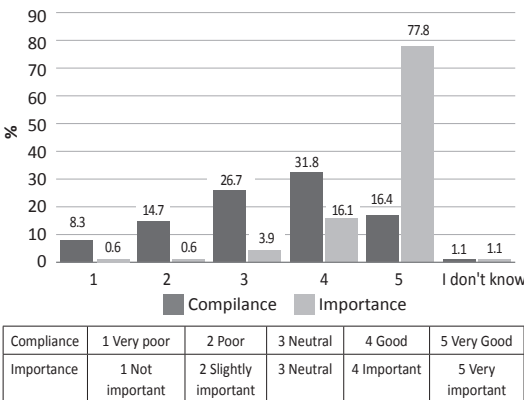


Figure 7. Assessment of the compliance and importance of Criterion 5 "Maintenance and appropriate enhancement of protective functions in forest management".

The vast majority of respondents (93.9%) assessed Criterion 5 as important and very important, with an average score of ratings being 4.59 (Figure 7). The results of Mann-Whitney U-test (Table A10 in the Appendix) for the level of importance showed that gender ($p=0.000$), age ($p=0.012$) and work experience ($p=0.040$) had a statistically significant influence on the average ratings. In general, female respondents, older respondents and respondents with more work experience gave higher average importance ratings for Criterion 5. The data in the latest Forest Europe report shows that no information was provided on forests with designated protective functions for BiH (Forest Europe 2020), and there is no available information on protective functions of forests in the official report (FMAWF 2022).

Figure 8 displays the distribution of responses given by respondents on the assessment of the compliance of Criterion 6 "Maintenance of other socioeconomic functions and conditions". This criterion is defined by analysing indicators/information on forest ownership, forest sector contribution to GDP, net revenue, investment in forests and forestry, forest sector workforce, occupational safety and health, wood consumption, trade in wood, wood energy, recreation in forests, and policies, institutions and instruments to sustain other socio-economic functions and conditions. Less than 40% of the respondents indicated that Criterion 6 was good and very good in compliance with forest management activities in FBiH, with an average score of 2.89 for the ratings. Further statistical analysis showed, as in all previous cases, that the differences between compliance ratings by independent variables were not statistically significant - Table A11 (see the Appendix).

When it comes to the assessment of the importance of Criterion 6, 89.5% of the respondents had the opinion that this criterion is important and very important, with an average value of ratings of 4.42. The results of Mann-Whitney U-test (Table A10 in the Appendix) for the level of importance showed that gender ($p=0.004$), age ($p=0.024$), work experience ($p=0.013$), and education ($p=0.002$) have statistically significant influence on average ratings. Accordingly, female respondents, older respondents, respondents with more work experience and those with a diploma from the pre-Bologna education system gave higher average ratings of importance for Criterion 6. According to data in the Ministry's official report, 4,825 people are employed in the forestry sector in FBiH, while data on the forestry sector's contribution to GDP, occupational safety and health, wood consumption, wood trade, wood energy and forest recreation are not available (FMAWF 2022). The comparison of differences in average scores was carried out to identify areas for further activities to improve the general understanding of the Pan-European criteria for SFM among FBiH forestry professionals. The average difference for the importance and compliance scores (1.60) was set as the breaking point for dividing the criteria into two groups: those where respondents expressed a low level of knowledge and understanding (>1.60) and those with the better level of knowledge and understanding (<1.60). Figure 9 shows the results of the comparison of the average scores for the compliance, importance and the differences

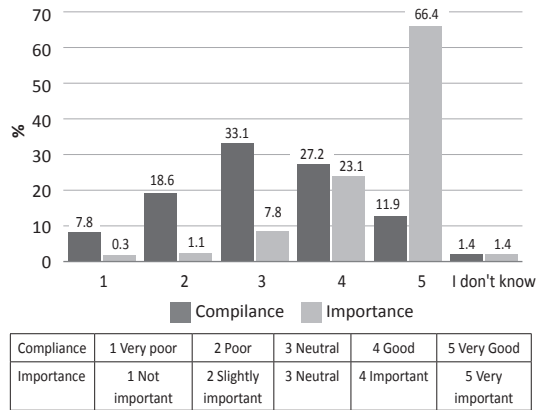


Figure 8. Assessment of the compliance and importance of Criterion 6 "Maintenance of other socioeconomic functions and conditions".

between ratings of six Pan-European criteria for SFM. The criteria with an average score difference of more than 1.60 are considered to be those where respondents expressed a low level of knowledge and understanding. Criterion 1, Criterion 2 and Criterion 4 belong to this category. On the aspects included in these criteria, specific forest policy instruments should be created focusing on continuous training, knowledge generation and information sharing among forest professionals in FBiH. In contrast, the criteria with an average score difference of less than 1.60 are treated as those for which the respondents indicated a better understanding. This group consists of Criterion 3, Criterion 5 and Criterion 6. Similar to the previous group, a specific forest policy instruments should be created to improve coordination between different institutional and market actors for information exchange and to make joint efforts to integrate the basic elements of Pan-European criteria for SFM in forest management activities in FBiH.

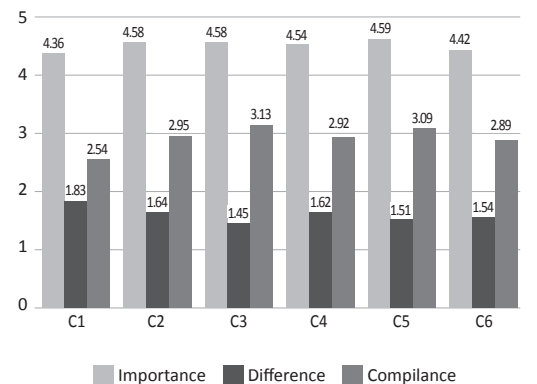


Figure 9. Average ratings of compliance and importance of six Pan-European criteria for SFM.

CONCLUSIONS

This study provides the findings of a survey of forestry professionals in FBiH to determine their attitudes and understanding of Pan-European criteria for SFM. The aspects of familiarity, compliance, and importance were explored, and an understanding of how the set of criteria might be utilised to enhance forest management activities in FBiH was elaborated. The general conclusion is that forestry professionals in FBiH have a relatively low level of familiarity with the Pan-European criteria for SFM, and that they express their low level of compliance with current forest management activities in FBiH, but they see the criteria as a useful and necessary tool for improving the forest sector in FBiH.

Considering the scope of the study, valuable data were obtained on the socio-demographic characteristics of forestry professionals in FBiH. According to the results, their average age is 41 years and their average work experience is just over 13 years. It can be said that this is a young population, most of whom will be working in the next 20 years or so. This kind of data is important for academic institutions when it comes to adjusting enrolment policies and also for labour market expectations and trends. As forestry professionals are a relatively young population, forest policy activities focusing on information and knowledge generation should be undertaken for them as a target group to improve their understanding of the Pan-European SFM criteria.

Concerning the low level of familiarity with the set of criteria, it can be concluded that there is a lack of understanding of international and European forest policy activities. This is understandable for the group of senior forestry professionals, who have not had the opportunity to study the subject of forest policy in the curriculum. On the other hand, these results show that adequate lifelong learning programmes in forestry need to be established and offered in FBiH. In addition to educational institutions (secondary forestry schools and forestry faculties),

professional associations (through the process of licencing forestry professionals for specific activities) should also play an important role in the continuous education of forestry professionals. Having in mind that the process of developing and applying Pan-European SFM criteria has been ongoing for almost three decades, it is clear that the exchange of experience, networking and coordination, focused on the implementation of these criteria in FBiH forestry, can improve current forest management activities and enrich the monitoring and assessment tools currently in use.

Since the majority of the respondents indicated that current forest management activities are insufficiently compliant with Pan-European criteria for SFM, significant changes to current forest policy activities in FBiH are required to adopt and integrate these tools into ongoing forest policy processes and forest management activities in general. Priority should be given to initiatives to educate and share information among all relevant stakeholders, especially forestry professionals responsible for monitoring and assessing progress in implementing the principles of SFM. It is also important to incorporate the requirements of the Pan-European criteria for SFM into the strategic and operational framework of forestry sector. In this respect, the relevant FBiH forestry institutions should initiate the joint process of developing new indicators and adopting existing set of SFM indicators. All administrative levels and forestry-related institutions should work together to carry out these activities. Furthermore, the implementation of the Pan-European SFM criteria in FBiH forestry requires the collection and analysis of data relevant to the specific indicators. Data for some of the quantitative indicators mentioned are already collected by the relevant institutions, while data for others are dispersed or missing altogether. In this regard, the database for analysing all relevant data on SFM activities, established in accordance with Pan-European criteria for SFM, is essential for their implementation, monitoring and reporting of the SFM activities in FBiH.

Appendix A

Table A1. Results of the Mann-Whitney U-test for the dependent variable Assessment of compliance of Criterion 1 Maintenance and appropriate enhancement of forest resources and their contribution to global carbon cycles.

Dependent variable: Assessment of compliance of Criterion 1 "Maintenance and appropriate enhancement of forest resources and their contribution to global carbon cycles"			
Independent variable	U	z	p
Gender: male and female	10550.000	-0.798	0.425
Age: up to 40 years and over 40 years	15191.000	-0.223	0.824
Work experience: up to 10 years, more than 10 years	15615.000	-0.290	0.772
Education: pre-Bologna system and Bologna system	12452.000	-0.254	0.799
Type of institution: forest company and public forest administration	6843.500	-0.776	0.438

Table A2. Results of the Mann-Whitney U-test for the dependent variable Assessment of importance of Criterion 1 Maintenance and appropriate enhancement of forest resources and their contribution to global carbon cycles.

Dependent variable: Assessment of importance of Criterion 1 "Maintenance and appropriate enhancement of forest resources and their contribution to global carbon cycles"			
Independent variable	U	z	p
Gender: male and female	9049.500	-2.893	0.004
Age: up to 40 years and over 40 years	13956.000	-1.667	0.095
Work experience: up to 10 years, more than 10 years	14720.000	-1.334	0.182
Education: pre-Bologna system and Bologna system	11004.000	-2.110	0.035
Type of institution: forest company and public forest administration	6871.000	-0.804	0.421

Table A3. Results of the Mann-Whitney U-test for the dependent variable Assessment of compliance of Criterion 2 Maintenance of forest ecosystem health and vitality.

Dependent variable: Assessment of compliance of Criterion 2 "Maintenance of forest ecosystem health and vitality"			
Independent variable	U	z	p
Gender: male and female	10899.500	-0.369	0.712
Age: up to 40 years and over 40 years	14553.500	-0.894	0.371
Work experience: up to 10 years, more than 10 years	15165.000	-0.755	0.450
Education: pre-Bologna system and Bologna system	12596.000	-0.088	0.930
Type of institution: forest company and public forest administration	7178.500	-0.268	0.789

Table A4. Results of the Mann-Whitney U-test for the dependent variable Assessment of importance of Criterion 2 Maintenance of forest ecosystem health and vitality.

Dependent variable: Assessment of importance of Criterion 2 "Maintenance of forest ecosystem health and vitality"			
Independent variable	U	z	p
Gender: male and female	9230.500	-2.751	0.006
Age: up to 40 years and over 40 years	13205.000	-2.625	0.009
Work experience: up to 10 years, more than 10 years	12951.000	-3.466	0.001
Education: pre-Bologna system and Bologna system	10357.000	-3.040	0.002
Type of institution: forest company and public forest administration	6087.000	-2.187	0.029

Table A5. Results of the Mann-Whitney U-test for the dependent variable Assessment of compliance of Criterion 3 Maintenance and encouragement of productive functions of forests.

Dependent variable: Assessment of compliance of Criterion 3 "Maintenance and encouragement of productive functions of forests"			
Independent variable	U	z	p
Gender: male and female	10996.000	-0.251	0.802
Age: up to 40 years and over 40 years	14489.000	-0.964	0.335
Work experience: up to 10 years, more than 10 years	14754.000	-1.182	0.237
Education: pre-Bologna system and Bologna system	11539.500	-1.309	0.191
Type of institution: forest company and public forest administration	6961.500	-0.598	0.550

Table A6. Results of the Mann-Whitney U-test for the dependent variable Assessment of importance of Criterion 3 Maintenance and encouragement of productive functions of forests.

Dependent variable: Assessment of importance of Criterion 3 "Maintenance and encouragement of productive functions of forests"			
Independent variable	U	z	p
Gender: male and female	9464.000	-2.385	0.017
Age: up to 40 years and over 40 years	14222.500	-1.391	0.164
Work experience: up to 10 years, more than 10 years	14362.500	-1.777	0.076
Education: pre-Bologna system and Bologna system	11130.500	-1.991	0.047
Type of institution: forest company and public forest administration	7299.000	-0.096	0.924

Table A7. Results of the Mann-Whitney U-test for the dependent variable Assessment of compliance of Criterion 4 Maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems.

Dependent variable: Assessment of compliance of Criterion 4 "Maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems"			
Independent variable	U	z	p
Gender: male and female	10588.000	-0.442	0.659
Age: up to 40 years and over 40 years	14343.000	-0.757	0.449
Work experience: up to 10 years, more than 10 years	15499.500	-0.058	0.953
Education: pre-Bologna system and Bologna system	12359.500	-0.141	0.888
Type of institution: forest company and public forest administration	5962.000	-1.808	0.071

Table A8. Results of the Mann-Whitney U-test for the dependent variable Assessment of importance of Criterion 4 Maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems.

Dependent variable: Assessment of importance of Criterion 4 "Maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems"			
Independent variable	U	z	p
Gender: male and female	8550.000	-3.289	0.001
Age: up to 40 years and over 40 years	13079.000	-2.322	0.020
Work experience: up to 10 years, more than 10 years	13918.500	-1.895	0.058
Education: pre-Bologna system and Bologna system	11148.000	-1.715	0.086
Type of institution: forest company and public forest administration	6553.500	-0.982	0.326

Table A9. Results of the Mann-Whitney U-test for the dependent variable Assessment of compliance of Criterion 5 Maintenance and appropriate enhancement of protective functions in forest management.

Dependent variable: Assessment of compliance of Criterion 5 "Maintenance and appropriate enhancement of protective functions in forest management"			
Independent variable	U	z	p
Gender: male and female	10558.500	-0.479	0.632
Age: up to 40 years and over 40 years	15026.000	-0.025	0.980
Work experience: up to 10 years, more than 10 years	15338.000	-0.228	0.819
Education: pre-Bologna system and Bologna system	12285.500	-0.228	0.820
Type of institution: forest company and public forest administration	6621.500	-0.787	0.431

Table A10. Results of the Mann-Whitney U-test for the dependent variable Assessment of importance of Criterion 5 Maintenance and appropriate enhancement of protective functions in forest management.

Dependent variable: Assessment of importance of Criterion 5 "Maintenance and appropriate enhancement of protective functions in forest management"			
Independent variable	U	z	p
Gender: male and female	8049.500	-4.087	0.000
Age: up to 40 years and over 40 years	12983.500	-2.511	0.012
Work experience: up to 10 years, more than 10 years	13835.000	-2.050	0.040
Education: pre-Bologna system and Bologna system	11420.500	-1.402	0.161
Type of institution: forest company and public forest administration	6204.000	-1.621	0.105

Table A11. Results of the Mann-Whitney U-test for the dependent variable Assessment of compliance of Criterion 6 Maintenance of other socioeconomic functions and conditions.

Dependent variable: Assessment of compliance of Criterion 6 "Maintenance of other socioeconomic functions and conditions"			
Independent variable	U	z	p
Gender: male and female	10851.500	-0.113	0.910
Age: up to 40 years and over 40 years	14489.000	-0.602	0.547
Work experience: up to 10 years, more than 10 years	14434.000	-1.181	0.238
Education: pre-Bologna system and Bologna system	11336.000	-1.340	0.180
Type of institution: forest company and public forest administration	6694.500	-0.675	0.500

Table A12. Results of the Mann-Whitney U-test for the dependent variable Assessment of importance of Criterion 6 Maintenance of other socioeconomic functions and conditions.

Dependent variable: Assessment of importance of Criterion 6 "Maintenance of other socioeconomic functions and conditions"			
Independent variable	U	z	p
Gender: male and female	8771.500	-2.897	0.004
Age: up to 40 years and over 40 years	13085.500	-2.252	0.024
Work experience: up to 10 years, more than 10 years	13359.000	-2.471	0.013
Education: pre-Bologna system and Bologna system	10023.500	-3.071	0.002
Type of institution: forest company and public forest administration	6613.000	-0.855	0.393

Author Contributions

BM, MA, SD conceived and designed the research, BM carried out the data collection, AB, BM and DŽB processed the data and performed the statistical analysis, MA supervised the research and helped to draft the manuscript, ŠPM helped to draft the manuscript, BM and MA wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Close-to-Nature Forestry Measures in East Polissia Region of Ukraine

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ABSTRACT

The article discusses close-to-nature forestry measures for the natural regeneration of pure and mixed pine forests. It is shown that successful natural regeneration of high value tree species takes place after uniformly gradual logging and progressive strip felling as final cutting operations in pure pine forests in fresh or moist oak-pine forest stands on sandy soil, resulting in the development of natural young pine forests with mixed composition. The article analyzes the state of natural regeneration after the first cycle of transformation felling operations in pure even-aged pine stands aimed at converting them into mixed pine forests of natural origin. Systems of close-to-nature silvicultural measures for restoration, development, improvement and regeneration of forest stands in the process of continuous cover forestry have been elaborated.

Keywords: felling; natural regeneration; development of mixed uneven-aged forest stands; biodiversity

INTRODUCTION

Close-to-nature forestry (CNF) is a system of organizational and forest management activities aimed at regeneration and development of natural forests. Natural forest stands are formed in accordance with forest types of the original stands with authentic seed origin, which is in accordance with the European Forest Management Strategy aimed at preserving continuous cover forestry (Mason et al. 2021).

For the regeneration and development of forest stands that are close to the original natural forests in terms of their species composition, age and spatial structures, forestry operations should involve environmentally-friendly technologies that contribute to preserving biotic diversity (Chernyavskiy et al. 2006, Krynytskyi et al. 2014).

Preservation and increasing species diversity is one of the fundamental principles of close-to-nature forestry that enhances forests' capacity to adapt to climate change and boosts their regeneration after the extreme negative impact of abiotic environmental factors (Schütz 1999, García-Güemes et al. 2014, Spathelf et al. 2015).

The natural regeneration of high value species occurs after clear, gradual and selective final felling operations (SFMCU 2010).

In the pine forests of North-East Germany, close-to-nature silviculture measures include uniformly gradual, group-selective felling and strip and wedge clearcutting (Lavnyy and Spathelf 2016).

Clearcutting (CC) is followed by the formation of mainly even-aged and temporary uneven-aged stands. The success of the process of natural regeneration is mainly influenced by the following factors: the area of the logging site, its width, the availability of seeding sources within the deforested area, as well as microclimatic, soil and hydrological conditions, cluttering with logging residues, condition and types of ground cover (Pogrebniak 1968, Vedmid et al. 2008, Lavnyy and Spathelf 2016, Zhezhkun 2021). In order to follow the principles of close-to-nature forestry, the maximum area of clear-cutting sites should be restricted. The maximum area allowed for clearcutting varies between European countries, with Germany, Austria, Italy and the Czech Republic restricting clearcutting to 1 ha and below, Poland (2 ha), Slovakia, Romania (3 ha) and

Bulgaria and Estonia up to 5 ha (Zasady 2003, Kravets and Kremenetskaya 2008, Lavnyy and Spathelf 2016).). In Ukraine, the area of clear felling is limited in pine forests up to 3 ha, and in oak forests up to 5 ha (SFMCU 2010). In the fresh and moist pine and pine-oak stands on sandy soil and clay soil in the eastern part of Polissia region of Ukraine, successful seed production leads to a satisfactory natural regeneration of Scots pine (*Pinus sylvestris* L.) and other valuable species with logging sites up to 70-80 m wide (Zhezhkun 2021).

Gradual and selective final felling systems belong to the selective form of forest management (Krynytskyi et al. 2012). After the uniformly gradual felling (UGF) in pine stands we can trace an increase in tree species diversity and structural diversity. Also, genetic intraspecific variability of trees is preserved and increased, and tree resistance to adverse biotic and abiotic factors (stresses) is enhanced, which corresponds to the principles of close-to-nature forestry (Brang et al. 2014, Krynytskyi and Chernyavskiy 2015). In the fresh and moist pine forest stands on sandy soils in the eastern part of Polissia effective results have been achieved in case of two-stage UGF and implementation of the soil cultivating measures aimed at supporting natural regeneration of Scots pine if carried out before seeding in a year of high seed production (Zhezhkun 2021). Gradual felling interventions aimed at the natural regeneration of high value tree species are carried out mainly in even-aged stands. In uneven-aged stands the communities of over-mature, mature, ripening, middle-aged and young forest generations grow on the same site. In order to maintain the dynamic balance and promote a developing multi-aged structure, selective felling operations with the removal of over-mature and mature trees are carried out (Tichonov et al. 2000, SFMCU 2010, Krynytskyi et al. 2012).

In recent years, against the backdrop of global climate change and increasing anthropogenic pressure, the sanitary condition of the forests has been deteriorating. Artificial forest stands and monocultures are characterized by low biotic stability, which leads to a decrease and suspension of growth processes, as well as to the weakening and drying-out of pine stands under the current conditions (Gilliam 2016, Meshchkova, Borisova 2018, Hlasny 2019, Klein 2020, Reuna et al. 2020, Zhezhkun et al. 2021).

In artificial pure even-aged stands, selection cutting (SC) is used to transform them into stable uneven-aged stands (Cabinet of Ministers of Ukraine 2007). In mountain conditions, the secondary forest stands of spruce, fir and beech are subject to transformation with a gradual shift to original fir-spruce-beech forest stands (Chernyavskiy et al. 2006, Krynytskyi et al. 2014). In even-aged artificial pine stands of homogenous composition in Eastern Polissia, the selective felling operations by forming small-scale forest logging sites and gaps ensure successful natural regeneration of Scots pine and common oak (*Quercus robur* L.), as well as other high-value tree species (Zhezhkun and Porokhniach 2017).

In uneven-aged oak forests, selective felling operations are offered among the close-to-nature silvicultural measures with the aim to reduce the canopy closure in parental forest stands, strip felling of undergrowth in a mast year, soil mineralization, and additional sowing of acorns (Chernyavskiy et al. 2006). Experiments have been conducted

on regenerated common oak stands' transformation into the forest stands of naturally regenerated or planted origin (Tkach 2010). Reproduction of natural pine stands in the steppe pinewoods of Ukraine is not linked to the final felling operations, so preservation of their biodiversity and genesis is carried out by complex felling methods, mainly by progressive felling in groups (Tkach et al. 2015).

Tending measures in the even-aged forests are carried out prior to the transformation felling in order to convert these forest stands into uneven-aged ones. In natural forests, tending begins with thinning, but is focused on fostering the target trees for the future (Krynytskyi et al. 2014, Lavnyy and Spathelf 2016).

To reduce the impact on natural environment during logging and other close-to-nature forestry activities, safe, environment-friendly logging technologies have been used (Vedmid et al. 2008, Krynytskyi et al. 2014, Lavnyy and Spathelf 2016). In the process of felling, fireless methods of cleaning the felling sites are used to avoid burning of the felling residues (Krynytskyi et al. 2014).

Thus, close-to-nature forestry includes a system of organizational and forestry tools for the restoration and formation of resilient, multi-age, complex-structured, mixed composition stands of natural origin. Felling and other forestry activities should be carried out using safe and sustainable technologies. Formation of uneven-aged stands with a multi-layered vertical structure accommodates the nature of shade-tolerant tree species; therefore, such an approach is widely practiced in mountain forests. In the region's lowland forests, experimental logging has started to be carried out for the natural regeneration of valuable tree species and the formation of native stands, which currently determines the scientific novelty and relevance of the research.

The purpose of the research is to study the results of experimental uniformly gradual felling and transformation felling operations to ensure a continuous forest cover and to develop a system of close-to-nature forestry measures in the Eastern Polissia region of Ukraine.

MATERIALS AND METHODS

The study was conducted in the forest reserves of state-owned forestry enterprises in the Eastern Polissia region of Ukraine (Chernihiv and Sumy regions) in the following types of forest ecological conditions: fresh and moist forest stand on sandy soil. In Ukraine, forest ecological conditions have a following trophic types: bor (pine or other coniferous forests, also mixed forests with a predominance of pine on very poor soils with oligotrophic vegetation), subor (types of forest ecological conditions characterized by relatively poor soils, close to "bor", but with an increased forest-vegetation effect), sugrud (types of forest vegetation conditions, combining relatively fertile soil conditions; the upper tree layer is formed by pine, oak, beech, spruce, fir, black alder and others), and grud (types of forest vegetation conditions that combine the most fertile habitat conditions; the upper layer is formed by oak, spruce, black alder, and less often beech and fir). Forest ecological conditions' typology by humidity includes: dry, fresh, moist, humid, wet (Pogrebniak

1968). In mature pine stands, gradual and continuous final felling operations were carried out for the natural restoration of Scots pine and other valuable tree species. Gradual felling was performed in two steps, and repeated every 3-5 years. In clearcuttings, the width of the logging sites ranged from 40 to 100 m, while the area of the logging sites was around 0.5–2.5 ha. The trees were felled with chainsaws and skidded in the form of debarked tree trunks or 4–6 m long segments using wheeled tractors along the skidding lines. Undergrowth of Scots pine and common oak was preserved on the logging sites after the gradual or continuous felling operations. After the first stage of gradual felling under the pine canopy which had been thinned down to the density of 0.4, and in the clearcutting sites the natural regeneration was promoted by cultivation of soil with disk cultivators or by making furrows with plows. The share of soil mineralization was 50–70% of the sites' area.

The transformation felling operations were implemented to convert even-aged pure pine stands into the mixed uneven-aged ones. The research plot with an area of 10 hectares is located in compartment #9 of the Slobidske Experimental Forest Unit of the Novhorod-Siversk Forest Research Station (Chernihiv region). A stand of artificial origin with the following characteristics was selected for transformation logging: stand composition – 9 PiSy1BePe, age – 66 years, average pine tree height – 24 m, average diameter – 28 cm,

density – 0.60, standing volume – 300 m³·ha⁻¹, forest type – fresh oak-pine subor. In the composition of the forest stand, the figures indicate the presence of each tree species (1 unit corresponds to 10% of the forest standing volume) and the abbreviated name of the tree species (PiSy – *Pinus sylvestris* L., BePe – *Betula pendula* Roth.). The sample plot is divided into 5 sections (2 hectares each) (Table 1). It was planned to carry out the transformation logging operations in 4 steps, repeated every 5-7 years with the transformation cycle of 15-20 years.

The experiment was designed in a way that the cycle of transformation felling involved removing part of the stand in small plots – from 0.045 to 0.25 ha within 1 ha logging site. In the first felling cycle two plots of 50×50 m each with an area of 0.25 ha were felled within 2 hectares of Section 1. On other sections, 1-5 gaps of different dimensions were formed evenly over the area. In subsequent felling cycles, two plots of 0.25 hectares were cut down within Section 1, and the gaps were expanded in other sections.

At each section, measures were tested to promote the regeneration of high value species (Table 2). For part of the logging site no interventions were implemented to promote natural regeneration (NR) and used for further comparison with the previously held measures. In the nomenclature of experiments, the number of the section and the measure to promote the regeneration were indicated (for example: 1-C).

Table 1. Parameters of the gaps formed as a result of transformation felling operations on the sample plot.

Section	Logging cycle (No.)	Gap dimensions (m)	Number of gaps per 1 ha	Area of gaps (ha)	Width of the forest regeneration site (thinning down to 0.4 density) (m)
1	1	50×50	1	0.25	-
	2	50×50	2	0.50	-
	3	50×50	3	0.75	-
2	1	diameter (D) 24*	5	0.23	-
	2	D 36	5	0.51	-
	3	D 44	5	0.76	-
3	1	D 36*	2	0.20	-
	2	D 42	2	0.28	-
	3	D 48	2	0.36	-
4	1	D 24*	3	0.14	12
	2	D 36	3	0.31	8
	3	D 44	3	0.46	5
5	1	D 36*	1	0.10	18
	2	D 44	1	0.15	22
	3	D 66	1	0.34	33

*Initial diameter of the gap after the first felling cycle: 24 m – 1 x stand height. 36 m – 1.5 x stand height

Table 2. Measures to promote regeneration of tree species.

Method of soil treatment	Depth of treatment (cm)	Mechanisms for soil treatment	Planting of seedlings (species, trees ·ha ⁻¹)	Variant code
Cultivation	5-7	Disc cultivator	-	R
Ploughing	10-12	Two-blade plough	-	B
Ploughing	10-12	Two-blade plough	PiSy -5,000 QuRo – 2,100	K

Inventory of self-sown seedlings (age – 1-2 years) and young trees (3-15 years) was held on the reference strips 2.5 m wide and 20 m long each. Those were 5-10 reference strips evenly distributed across the plot. The inventory was held for 2-5% of the plot surface. In the process of accounting for undergrowth and self-sown trees, the species composition, origin, age, viability, density, and success of natural regeneration were determined according to the current scales (Pasternak 1990, Forester's Manual), amended and restated (Zhezhkun 2021). In particular, for the natural regeneration of 4-8 years old Scots pine, the amount of viable undergrowth (trees per 1 ha) is divided into following categories: "good" – more than 6,000, "satisfactory" – 3,000-5,999, "insufficient" – 1,000-2,999, "bad" – less than 999 (Table 3).

Permanent sample plots were laid in accordance with the requirements (Sample plots of forest inventory 2006) after the canopy in regenerated young stands got closed. The determination of taxation specifications of the stands within the permanent sample plots was carried out using officially adopted manuals and guidelines (Kashpor and Stochynskiy 2013). In the composition of stands, the admixture of arboreal species (3-5% of the standing volume) was marked with a "+" sign, and the admixture of 1-2% of the standing volume was marked with the "unit" index. The research materials were processed by applying mathematical and statistical methods using software Microsoft Excel (2019). We determined the probability of differences in the average amounts of undergrowth and self-sown individuals of tree

species by experimental variants, and compared the amount of undergrowth and self-sown trees with the standards provided in the rating scale for assessing the success of natural regeneration.

RESULTS

The first cycles of gradual felling in pine stands were carried out in the period 2009–2014. The intensity of the first stage of GF was 122–223 m³ha⁻¹ (30–52% of standing volume). The density of pine stands was reduced to 0.3–0.4. Given a successful mast seeding of Scots pine in the first year, 10,000–35,000 trees·ha⁻¹ regenerated. For 4–5 years, the density of pine undergrowth was 8,000–20,000 trees·ha⁻¹ and 2,000–9,000 trees·ha⁻¹ of 1–2-year self-sown trees. The frequency of pine undergrowth occurrence was 73–84%, and the placement on the plots was uniform. During the final stage of GF, the preservation of undergrowth was 71–96%. The composition of young stands formed after gradual felling was dominated by Scots pine (Table 4). The canopy closure was 0.26–0.4, which indicated the formation of special forest environment (Shvidenko and Ostapenko 2001). The largest number of pine trees was observed on the experimental plots No. 2, No. 4 and No. 5 in dense bio-

groups. The share of deciduous species in young forest stands was 10–40%, which was sufficient for the formation of mixed stands of natural origin.

Table 3. Rating scale for natural regeneration success of tree species (Zhezhkun 2021).

Success category	Quantity of viable seedlings and saplings per 1 ha by age				
	1 year old	2 years old	3 years old	4-8 years old	0-16 years old
I. Good	> 40001	> 20001	> 12001	> 6001	> 4001
II. Satisfactory	26001 - 40000	10001 - 20000	7001 - 12000	3001 - 6000	2001 - 4000
III. Insufficient	15001 - 26000	6001 - 1000	3001 - 7000	1001 - 3000	501 - 2000
IV. Bad	< 15000	< 6000	< 3000	< 1000	< 500
Conversion factor to 4-8 years old	0.2	0.3	0.7	1.0	1.5

Table 4. Forestry and taxation indicators of young stands of natural origin formed after gradual and final clearcutting.

Plot No.	Felling type	Stand composition*	Average age (years)	Average height (m)	N (trees·ha ⁻¹)		Canopy density
					Total	Pine	
1	UGF**	7PiSy1PoTr1BePe1QuRo	5	1.0	11,700	7,600	0.30
2	UGF	8PiSy1BePe1QuRo	6	1.4	15,700	12,900	0.34
3	UGF	6PiSy3BePe1QuRo	7	1.8	6,300	4,600	0.26
4	UGF	7PiSy2BePe1PoTr+QuRo	7	1.7	25,900	17,900	0.40
5	UGF	9PiSy1BePe+QuRo	7	1.8	17,500	15,700	0.36
6	CC***	5PiSy3PoTr2BePe+QuRo	5	0.7	11,400	5,400	0.40
7	CC	10PiSy+BePe,1Potr	6	1.0	11,400	10,000	0.30
8	CC	7PiSy3BePe, 1QuRo	7	1.4	7,100	4,900	0.30

* BePe – *Betula pendula* Roth., PiSy – *Pinus sylvestris* L., PoTr – *Populus tremula* L., QuRo – *Quercus robur* L.

** UGF – uniformly gradual felling

*** CC - clearcutting

Satisfactory regeneration of Scots pine occurs after final clearcutting with soil mineralization of 50–60% of the plot area (plot 6). The proportion of deciduous species increases up to 50% from the density of a 5-year-old stand. Pine trees are suppressed by common aspen (*Populus tremula* L.) and silver birch (*Betula pendula* Roth.), and this situation requires tending interventions. On the plots 7 and 8, most of the natural regeneration of pine (83–92%) is located in former plough furrows, 1.4 m wide, laid at a distance of 2.5 m. The availability of deciduous species among the young stand composition will enable to form natural pine stands of mixed composition.

Intensity of the stage of transformation logging cycle carried out within the sections in 2009–2010 was made up from 53 to 105 m³·ha⁻¹ or from 16.8 to 40.2% of the standing volume. On average, the felling intensity compared to the standing volume on the plot was 71.2 m³·ha⁻¹ or 24.1%, which corresponded to the standard provided by the Regulations on Improving the Quality Composition of Forests (CMU 2007). Given the average yield of Scots pine mast in the first year, 3,300 to 60,500 seeds·ha⁻¹ of self-sown trees regenerated in the gaps. Even in the variant that does not involve assistance to natural regeneration in Section 1, the density of 1-year old self-sown pine was 11,600 trees·ha⁻¹, which was explained by partial mineralization of the soil during felling operations. In variants that involved planting of pine and oak trees during the first year, the survival ability was 77–94%.

Over the next 5 years, the density of natural regeneration of Scots pine on the plots with furrows was 9,500–21,000 trees·ha⁻¹. In variants where the soil was leveled with a cultivator, the density of natural pine regeneration was slightly lower than 7,500–18,100 trees·ha⁻¹. On the plots without assistance to natural regeneration, the success rate of natural pine regeneration was satisfactory as well (2,500–6,000 trees·ha⁻¹). In the variants that envisaged planting of pine and oak trees, the density was 3,400–6,900 trees·ha⁻¹ and the natural regeneration of pine was 1,100–9,600 trees·ha⁻¹.

The most successful natural regeneration after the first cycle of transformation logging operations was observed within the gaps whose diameter is equal to the stand's height (Section 2) and on one height of the stand with thinning on the forest regeneration site (0.5 of the gap diameter) with the density of 0.4 (Section 4). On such plots, subject to partial tending of the soil with furrows, the amount of natural regeneration of pine when applying the conversion factor to 4–8 years old amounted to 14,100–21,300 trees·ha⁻¹, and in case of cultivation with the cultivator, 11,100–12,000 trees·ha⁻¹.

Stands in the Sections 2, 3, and 5 were damaged by heavy wind storms in the summer of 2014. It should be mentioned here that the most significant increment and the smallest die-off compared to the standing volume was observed during 5 years after the first cycle of transformation logging on the Sections 2 and 4 (respectively: 37.4 m³·ha⁻¹ and 43.0 m³·ha⁻¹, and 15.9 m³·ha⁻¹ and 0 m³·ha⁻¹), compared to Sections 3 and 5 with the gap size of 1.5 of the average stand height (26.7–32.9 m³·ha⁻¹, and 21 and 18 m³·ha⁻¹). In Sections 3 and 5, the mortality exceeded the indicators of the current periodic change in the standing volume after the 1st felling cycle. Therefore, in order to maintain the biotic stability of pine stands intended for transformation felling, the diameter of a gap should be limited to one average height of the stand. Dead

trees were removed by selective sanitary felling in 2014.

The regeneration of tree species occurs not only in the gaps formed in the first cycle of transformation felling, but also between the gaps and under the stand canopy (200–600 trees·ha⁻¹). Reforestation in the sections took place not only with Scots pine, but also with tree species that were not desirable for restoration (common aspen (*Populus tremula* L.), goat willow (*Salix caprea* L.), silver birch (*Betula pendula* Roth.) and others). To improve the vegetation conditions for the pine trees in previous years, grass cover and unwanted tree and bush species were cut down with a bush cutter.

The second cycle of transformation felling was implemented in 2016, and the measures to assist regeneration were carried out in the spring of 2017. The felling intensity in the sections was 52–105 m³·ha⁻¹ or 21–37% of the pine stands' standing volume. Simultaneously with the clear-cutting elements, the undergrowth and young tree stands were thinned on the plots where the previous felling cycle had been held. All undesirable trees and shrubs that shaded the Scots pine and common oak trees were removed. During the period 2017–2020, the standing volume increment decreased to 7–15 m³·ha⁻¹ (mortality - 1–2 m³·ha⁻¹) compared to the period after the 1st cycle of felling, which was explained by a decrease in the density of thinned stands. The amount and success of regeneration within the sections during 2017–2020 was different (Table 5).

Section 1 with plough furrows is dominated by natural regeneration of pine trees aged 4–8 years. The regeneration of 1–2-year self-sown trees took place. Therefore, the success rate of regeneration is good. In the variant that envisaged cultivation of soil with a disk cultivator (1-C), the density of pine is slightly lower than in the variant 1-F. A certain obstacle to regeneration and growth of self-sown pines is competition with abundant ground cover. The success rate of natural regeneration of Scots pine is satisfactory.

In Section 2, in the variant with the creation of forest plantations (2-P), the success rate of regeneration has improved over 5 years and is estimated as good (see Table 4). Other variants were also characterized by the improvement in regeneration success (mostly up to the satisfactory rate). In the variant with thinning operations held at a wrong time (2-C), regeneration is rated as insufficient. In the variant with natural regeneration 2-NR (without any human assistance), its success rate has somewhat improved, but is still insufficient.

In Section 3 during 5 years after the 2nd cycle of transformation felling operations, the regeneration progress has mostly been insufficient. Only in the section with the furrows (3-F) it is rated as satisfactory. The deterioration in regeneration success was due to a decrease in the amount of thinning and the dieback of Scots pine (especially in variant 3-NR without measures aimed at assistance to regeneration).

In Section 4, the regeneration progress is rated as satisfactory, except for the option 4-C with cultivation of the soil with a disk cultivator. Deterioration in the regeneration success also occurred due to insufficient treatment for high value species.

In Section 5, with the largest diameter of gaps, the regeneration progress is rated as satisfactory given all the needed assistance and control. This fact is explained by preserving the pine undergrowth, which appeared in the

Table 5. Number of trees of natural and artificial origin that regenerated after the second cycle of transformation felling in 2017-2020.

Section and variant No.	Amount of natural common pine regeneration by age (trees·ha ⁻¹)					Density of artificial regeneration (trees·ha ⁻¹)		Regeneration rating
	1	2	3	4-8	Total	Pine	Oak	
1-C	–	670	260	3,970	4,900	–	–	satisfactory
1-F	200	4,000	510	8,900	11,610	–	–	good
2-P	–	–	210	4,850	5,060	2,800	–	good
2-NR	–	–	250	1,510	1,760	–	–	unsatisfactory
2-F	260	510	1,030	4,870	6,670	–	–	satisfactory
2-C	–	500	250	2,000	2,750	–	–	unsatisfactory
3-C	670	–	670	2,000	3,340	–	–	unsatisfactory
3-F	7,810	2,500	1,090	1,870	13,270	–	–	satisfactory
3-NR	5,000	–	–	400	5,400	–	–	unsatisfactory
3-P	670	1,330	130	400	2,530	130	–	unsatisfactory
4-F	2,870	1,380	1,250	3,500	9,000	–	–	satisfactory
4-C	750	–	500	2,000	3,250	–	–	unsatisfactory
4-P	960	340	–	1,580	2,880	1,230	620	satisfactory
5-P	2,420	1,900	530	100	4,950	3,210	–	satisfactory
5-F	4,500	1,400	900	2,800	9,600	–	–	satisfactory
5-C	1,670	–	–	4,000	5,670	–	–	satisfactory
5-NR	1,670	–	–	4,000	5,670	–	–	satisfactory

C – cultivation, F – Ploughing, P – plantations, NR – natural regeneration

regeneration strip after the first cycle of transformation felling. With an increase in the amount of pioneering regeneration and its preservation after the second cycle of felling, the amount of regeneration of softwood species decreases, which contributes to the successful growth of pine.

Thus, the best results of regeneration after the second cycle of transformation felling were obtained in the sections with the largest gap dimensions (1.5–2 height of the stand). The success of regeneration may deteriorate due to untimely thinning of pine and other valuable species.

The third stage of transformation felling was held in 2021. The logging operations intensity in the sections was 65–94 m³·ha⁻¹ or 28–45% of pine standing volume.

The first stage of transformation felling in 2009 resulted in the formation of 11-year-old mixed pine stands on the plots (Table 5). It is worth noting that in 2016, the second stage of restocking felling was followed by thinning of the young stands regenerated in the gaps from the first felling cycle. During the thinning operations, all the viable trees of Scots pine, common oak, European spruce, small-leaved lime were left untouched. The unwanted specimens of marginal species (silver birch, goat willow, aspen, and common hazel) were cut down. Individual specimens and groups of silver birch trees were left only in places where more valuable species did not regenerate. Therefore, the composition and structure of the newly formed young stands were somewhat different and depended on the success and abundance of regeneration with Scots pine and common oak, as well as on timely thinning.

11-year-old mixed pine young stands were formed on the plots after the first cycle of transformation felling

operations carried out in 2009 (Table 3). It is worth mentioning that in 2016 in young stands regenerating in the gaps left from the first cycle of felling the thinning measures were implemented after the second cycle of transformation felling. During these thinning interventions all viable individual trees of Scots pine, common oak, Norway spruce (*Picea abies* (L.) Karsten), and small-leaved lime (*Tilia cordata* Mill.) were left. The unwanted trees of marginal species (silver birch, goat willow, aspen, common hazel (*Corylus avellana* L.) were removed. Individual silver birch trees and its groups were left intact only in the places without regeneration of more valuable species. Therefore, the composition and structure of the newly formed young stands were somewhat different and depended on the success and abundance of regeneration with Scots pine and common oak, as well as on timely applied thinning interventions.

In Section 1, with the removal of trees in the logging sites with the dimensions of 50×50 m (0.25 ha), Scots pine prevails in young stands in the variant with no assisted regeneration (1-NR). On some of the plots, the birch trees exceed the pines in height, which required regulating their interconnection by thinning. In a young stand characterized with high rates of standing volume, common oak trees (836 trees·ha⁻¹) are inferior in average height to almost all the other species (difference of 0.4–1.0 m or 21–52%). To form a mixed oak-pine stand with an admixture of small-leaved lime in a fresh oak-pine subor (forest on relatively poor soils, but with an increased forest-vegetation effect), it is necessary to undertake cutting of unwanted species. As a measure of treatment, the trees of marginal species that shaded common oak and Scots pine were removed during thinning.

Table 6. Forestry and taxation indicators of mixed pine stands formed after the 1st cycle of transformation felling operations.

Variant code	Composition*	Age (years)	Average height (m)	Average diameter (cm)	Sum of cross-sections (m ² ·ha ⁻¹)	Density	N (trees·ha ⁻¹)	Standing volume (m ³ ·ha ⁻¹)
1-F	3PiSy6BePe1QuRo, singly PoTr, TiCo, AcPl	11	2.0	1.1	2.39	0.78	20 100	7.4
1-C	2PiSy6BePe1QuRo1PoTr	11	2.1	1.5	1.72	0.37	9 800	5.4
2-P	Forest crops 9 PiSy1QuRo	11	2.7	2.3	1.20	0.31	3 620	3.3
	Natural regener. 8 PiSy2 BePe, singly SaCa, QuRo, PoTr	11	2.8	2.5	2.47	0.48	21 377	7.1
2-NR	7QuRo1PiAb1PiSy1SaCa, singly PoTr, BePe	11	3.0	3.5	1.73	0.26	4 697	4.1
2-F	5PiSy4BePe1QuRo, singly SaCa, AcNe, PoTr	11	2.8	2.4	5.60	0.91	21 043	17.2
2-C	3PiSy4BePe3PoTr, singly QuRo, SaCa	11	1.6	0.8	0.63	0.17	11 500	2.0
3-1C	3PiSy5BePe1QuRo1SaCa, singly PoTr	11	2.2	1.7	1.61	0.43	10 856	4.8
3-2F	10BePe+QuRo, singly SaCa, PiSy, PoTr, MaSy	11	3.6	2.4	3.75	0.47	8 879	13.3
3-NR	4PiSy6BePe+ QuRo, singly PiAb, MaSy,PoTr, RoPs	11	2.6	2.1	2.69	0.38	5 434	8.5
3-P	Forest crops 9PiSy1QuRo	11	3.5	3.5	1.52	0.18	2 915	4.5
	Natural regener. 7BePe2QuRo1PiSy, singly PoTr	11	2.7	1.4	2.43	0.41	11 574	7.5
4-F	3PiSy3QuRo4BePe, singly PiAb, SaCa, PoTr	11	2.4	1.9	3.23	0.58	16 819	9.4
4-C	5PiSy3BePe1QuRo1SaCa, singly PoTr	11	2.3	1.8	2.21	0.52	10 715	6.9
4-P	Forest crops 10PiSy+QuRo	11	3.5	3.6	2.96	0.33	3 968	7.7
	Natural regener. 5PiSy3BePe2QuRo+SaCa, PoTr	11	2.8	2.4	2.06	0.39	12 221	5.8
5-P	Forest crops 8PiSy2QuRo	11	3.5	3.5	0.80	0.09	1 081	1.8
	Natural regener. 7BePe2PiSy1QuRo, singly PoTr	11	2.3	1.0	0.32	0.08	3 243	1.1
5-F	5PiSy5BePe+SaCa, singly QuRo, PoTr	11	3.2	3.0	2.10	0.38	9 257	5.7
5-C	6PiSy3BePe1QuRo+SaCa, singly PoTr	11	3.1	2.8	6.39	0.99	15 500	19.8
5-NR	7BePe1PiSy1QuRo1SaCa+PoTr, singly PyCo	11	4.8	4.0	6.24	0.79	7 332	22.3

* AcNe – *Acer negundo* L., AcPl – *Acer platanoides* L., BePe – *Betula pendula* Roth., MaSy – *Malus sylvestris* Mill., PiAb – *Picea abies* (L.) Karst., PiSy – *Pinus sylvestris* L., PoTr – *Populus tremula* L., PyCo – *Pyrus communis* L., QuRo – *Quercus robur* L., RoPs – *Robinia pseudoacacia* L., SaCa – *Salix caprea* L., TiCo – *Tilia cordata* Mill.

In variant 1-F (assistance to natural regeneration by furrowing), the average height of Scots pine is 0.2 m (10%) less than that of birch. Birch (15,200 trees·ha⁻¹) and aspen (200 trees·ha⁻¹) trees inhibit the growth of Scots pine trees (3,900 trees·ha⁻¹) and common oak (500 trees·ha⁻¹). In order to regulate the interconnection between the trees in the stand, purging treatment is recommended.

In variant 1-C (cultivation of the soil with a disc cultivator), the share of Scots pine amounts to 22% of the young forest standing volume. Pine density (2200 trees·ha⁻¹) remains sufficient for the formation of pine with an admixture of common oak (800 trees·ha⁻¹). The dieback of pine trees has been detected (100 trees·ha⁻¹) due to the suppression of birch and aspen trees that require improvement felling.

In Section 2 with the size of gaps of 24 m in diameter, the highest rated regeneration progress was observed in the variant 2-P where pine and oak plantations were created. The number of pine trees of natural origin is 1.6 times higher than the density of planted stands with the same species, and not inferior in average morphometric indicators of height and diameter. Oak trees of both artificial (1 000 trees·ha⁻¹) and natural (520 trees·ha⁻¹) origin are inferior in average height to other species, and are suppressed by them. Thinning is recommended to regulate the interconnection between the trees. In the variant 2-F with furrow lying, the Scots pine

also dominates in the composition of young stands. The share of Scots pine is somewhat small (30% of the stock) in the variant with soil cultivation (2-C). The interconnection between pine, oak and soft-leaved trees should be regulated via selection logging operations.

In Section 3 with a gap size of 36 m after the first felling cycle in the variant with soil cultivation (3-C), pine is inferior in terms of standing volume (31%) to birch (45%). The difference in the average height of the compared species is insignificant (0.2 m or 9% for birch), which is explained by timely maintenance. However, in the adjacent gap (3-F), birch is completely dominant. Pine showed poor regeneration success and died in the first years due to oppression by birch. The site also demonstrates the consequences of untimely treatment of high-value species (pine and oak density: 0.09 and 0.17 thousand pcs. ha⁻¹, respectively). Even in the 3-NR variant which included natural regeneration (without any assistance to regeneration, but with partial removal of litter during skidding), the share of pine in the composition is 37% of the standing volume. The average height of a birch tree (5.5 m) is more than twice that of a pine tree, so tending logging operations are recommended. As in Section 2, the best composition in terms of high value species was found in the variant where pine and oak plantations were created (3-P). The availability of an admixture of pine and

oak trees of natural origin in the composition of the young stands enables the formation of a stand of highly valuable composition and combined origin.

In Section 4, due to the size of gaps of 24 m in the diameter and the thinning operations held in the regeneration strip for all the variants, there is predominance of 11-year-old young stands of Scots pine and common oak. The largest number of pine trees of natural origin was preserved in variants with furrowing as a measure to assist the regeneration process (4-F) and in plantations (4-P). Timely held tending (improvement) felling may lead to the formation of young mixed pine stands of natural and combined origin.

In Section 5 with the diameter of gaps of 36 m and thinning being held in the regeneration strip for 11 years since the first cycle of restocking felling, the predominance of Scots pine in the composition of young stands was revealed in the variants with assisted regeneration using furrows (5-F), cultivation with a cultivator (5-C) and by establishing plantations (5-C). In this section, the average height of pine trees of natural origin is the highest compared to other sections. Only in the variant 5-NR without any assistance to regeneration, but with the partial removal of litter during skidding, the share of Scots pine is 11% of the standing volume. The composition is dominated by silver birch, which exceeds the average height of pine by 1.7 m (55%) and suppresses its growth. Therefore, assistance to the natural regeneration of Scots pine and common oak (*Quercus robur* L.) is the most important measure for the reproduction of high value forest stands.

Thus, during the period of 11 years after the first cycle of transformation logging, young pine forest stands of mixed composition both of natural and combined origin were formed on the sections. The standing volume of young stands ranges from 2 to 22 m³·ha⁻¹, and the average increment is 0.2–2.0 m³·ha⁻¹. The density of young stands is different, ranging within 0.17–1.03, which is explained by different rates of regeneration progress and preservation status of the trees of the main forest-forming species, as well as timely held felling treatment aimed at forest stand improvement.

After the second cycle of transformation felling, pine and oak regeneration progress in the variants with assistance to natural regeneration and establishing plantations is mostly satisfactory, and in the sections without assistance it is insufficient. The success of the main forest-forming species preservation and forming young forest stands will also depend on the timely held tending operations - improvement felling.

After the third cycle of the transformation felling, the best rate of the natural regeneration was observed in Section 1 in the variant with natural regeneration (1-NR). As of 18 October 2021, the density of annual self-seeding of Scots pine was 10,800 trees·ha⁻¹, aspen – 10,000 trees·ha⁻¹, and goat willow – 6,000 trees·ha⁻¹. Individual common oak and white birch were found in the admixture. Regeneration success rate was good. In other variants an additional clearing of logging sites, combustion of logging residues, implementation of measures to promote the restoration of pine and oak were ongoing during the study.

Until the final fourth stage of restocking felling is carried out, the increment will increase for the young stands on the sections used in the previous cycles. Gradual regeneration will enrich the age diversity and preserve the biotic diversity. The combination of elements of final felling for the older generation of pine stands and the improvement felling operations for the valuable tree species of the new generation meets the principles of comprehensive logging.

Comprehensive transformation logging gradually converts an artificial even-aged weakened pine stand into a stable uneven-aged mixed pine forest stand of natural origin, which is coherent with the principles of close-to-nature forestry. After the completion of the transformation logging cycle, it is recommended to recreate a stand with the following structure: 70–80% of Scots pine, 10–20% of common oak, 10–20% of associated species, aged 3–20 years, with a cascade profile of the canopy (due to gradual regeneration within the gaps), and homogenous distribution of the trees within the site.

DISCUSSION

Forest management should ensure the continuous existence of forest cover. In the mature forest stands in Ukraine, four systems of final felling are currently utilized (State Forest Management Committee of Ukraine 2010) (Figure 1).

The most common is the final clearcutting system. Gradual felling may be carried out in the pine stands on 20–30% of their area (Vedmid et al. 2008, Zhezhkun 2021). Selective felling operations are carried out in uneven-aged stands (Tichonov et al. 2000, Krynytskiy et al. 2012), but their area in the Polissia region of Ukraine is restricted (Hensiruk 2002, Vedmid et al. 2008). The combined logging system that unites different types of final felling is rarely used (Tokarieva 2021).

When implementing different felling systems, one should apply fireless methods of clearing felling sites. In particular, in dry and fresh hygrotopes, it is advisable to shred the logging residues and coat the soil to provide shade from the direct light, and in wet and damp hygrotopes – to leave them to decay. Fire safety measures are used to prevent fire. It is allowed to process large-sized felling residues into technological chips for further sale, but the thin branches should be left to decay so that the soil fertility and biodiversity are ensured.

After logging and purging activities are carried out, measures are taken to promote the regeneration of high-value species. The viable undergrowth of Scots pine, Norway spruce (*Picea abies* (L.) Karst.), common oak, common ash (*Fraxinus excelsior* L.) and other species has to be preserved. To facilitate the natural regeneration process of the valuable species, the soil is mineralized prior to the mast year by cultivation with disk cultivators and harrows, and by plowing furrows. According to the studies, the share of soil mineralization for a successful natural regeneration should be more than 60% of the site area. In the years with poor seed yield, seeds of the main forest-forming species are sown (in particular, common pine – 1 kg·ha⁻¹). In case of

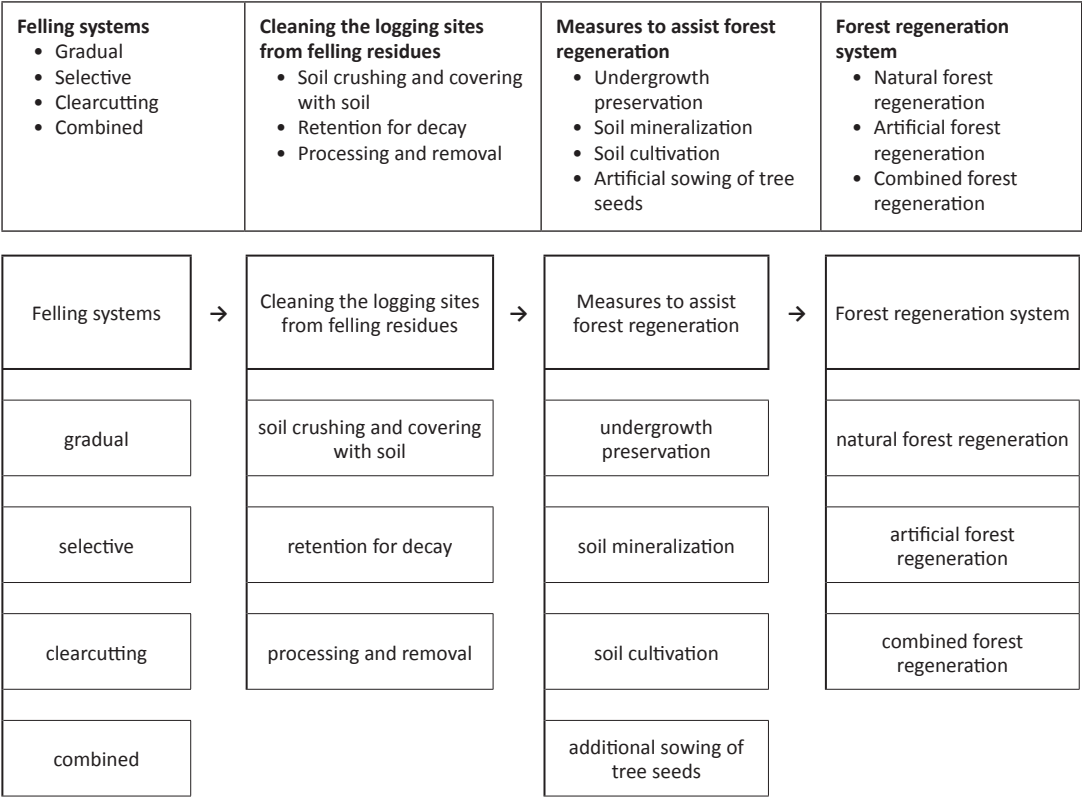


Figure 1. Final felling and forest regeneration systems for close-to-nature forestry.

the impossibility to ensure natural regeneration, the soil is cultivated for artificial reforestation.

In the pine forest stands, the most effective measures to ensure natural regeneration are as follows: the uniformly-gradual logging, gradual logging in strips, gradual felling in groups, and clearcutting in strips. In the pine forests of fresh and wet subors (on poor soils), after the double-stage uniformly-gradual logging, from 8,000 to 20,000 trees·ha⁻¹ of 4–5 year-old Scots pine trees are regenerated, which is sufficient to form the high-value forest stands. During the 4–5 year cycle of the uniformly-gradual logging operations, the undergrowth is preserved, so the pine and other species regenerate simultaneously, which contributes to the formation of relatively uneven-aged stands (Figure 2).

After the strip-shaped gradual logging and clearcutting on the logging sites up to 80 m wide, the successful regeneration of Scots pine (density of 5,000–10 ,000 pcs. ·ha⁻¹, which allows to schedule the adjacent logging sites for felling in 4–5 years) has been ensured. However, in the context of close-to-nature forestry, the scope of clear-cutting should be reduced, in accordance with the studies (Tokarieva 2021), and the area of the logging sites should be limited to 0.5–1 ha. If the undergrowth is unevenly distributed, then successful natural regeneration process

occurs after three rounds of gradual felling by groups (Zhezhkun 2021). In addition to Scots pine, the natural regeneration involves common oak, silver birch, and small-leaved lime trees, which will facilitate the onset of the mixed stands with high biotic stability and productivity.

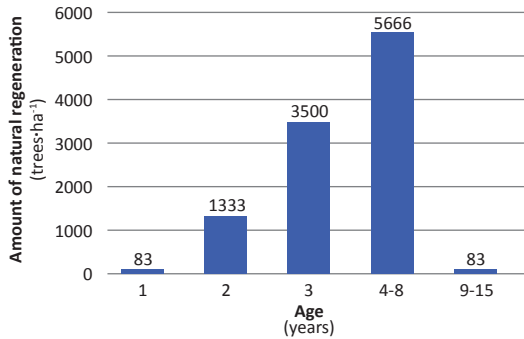


Figure 2. Age structure of the undergrowth and self-sown trees of Scots pine 2 years after the final stage of UGF (compartment 40, sub-compartment 10 of the Horodnianske Forestry Unit, Chernihiv region).

Artificial regeneration is carried out only in case of poor natural regeneration of high-value tree species. The development of forest cultures is carried out by planting trees or sowing seeds of either principal or related species to form mixed biologically resistant stands.

Given the partial retention of trees from natural regeneration of the forest, the vacant areas within the plots are planted or sown with trees for the combined forest stands' regeneration process.

In the process of stand development, the internal relations between the trees are regulated by tending felling operations (Figure 3).

Improvement felling is carried out in order to shape the species composition, regulate the density, enhance the ecological conditions for the chosen trees, increase sprouting, as well as improve marketability, and other quality indicators of the remaining trees (Cabinet of Ministers of Ukraine 2007). In particular, canopy opening interventions are carried out in young stands up to 10 years of age to create the desired composition and density of the stand. Early thinning is carried out in young forests of 11-20 years of age to ensure the composition and homogeneous distribution of the main forest-forming species of trees over the site, to shape the optimal structure of the future forest stand, and to regulate the quantitative ratio of individual tree species. In the middle-aged stands, thinning is carried out to maintain the shape of the trunk and crown of the finest trees, while severance felling is conducted to increase the increment of the best trees, improve their marketability, and enhance the resilience of the stands.

Depending on the age, composition, and density, moderate or high intensity improvement felling operations are performed. Untimely maintenance or poorly-performed improvement logging leads to the development of secondary stands with the predominance of species that do not meet forest vegetation conditions or, conversely, stands of homogenous composition characterized by low biotic stability originate. It is possible to correct the secondary and

low-value young forest stands by reconstructive logging: introducing the main forest-forming species into their composition and maintaining them using the developed technologies (Zhezhkun 2021). It is important to note that in previous years, the research area was dominated by the development of pure monocultures of Scots pine, Norway spruce, common oak, etc. (Zhezhkun 2021).

The resulting mono-dominant even-aged stands have low biotic stability and in years with unfavorable factors are affected by pests and diseases. They are also subject to massive drying-out and decline. The transformation of pure even-aged cultures into mixed stands of diverse age groups is carried out via transformation logging. At the age of maturity of such stands, the forest restoration felling is carried out.

To ensure timely recovery of the forest stands, the removal of the trees freshly invaded by pests and the placement of trapping trees is applied. In particular, placement of trapping trees is carried out by cutting down and leaving seriously weakened trees of a certain species on the site, which are used as traps for bark beetles, and then removing them from the forest. In case of stand disruption due to the damage caused by squally winds, snow storms, fires, etc., felling is carried out to localize and eliminate the consequences of the disasters. In particular, logging of large burnt areas refers to logging on sites with an area of more than 5 hectares, where stands were destroyed by a forest fire.

Close-to-nature silviculture in forests of different functionality and purposes has its own peculiarities. In forests of high nature conservation value, the system of measures of close-to-nature forestry should be aimed at strengthening the conservation functions of reserves, sanctuaries and other protected areas. In the case of protective forests, it should be aimed at improving water-regulating and soil-protective properties. In recreational forests, the aim is to increase the attractiveness of forest and park landscapes while maintaining the biotic stability

Tending logging systems	Sanitation felling systems	Transformation and reforestation felling systems	Felling systems to mitigate the consequences of calamities
Canopy opening	Felling of the trees with early signs of pest invasion	Transformation felling	Logging of windthrow / windstorm sites
Clearing (?)	Laying trapping trees	Transformation felling	Salvage logging and clearance on wildfire sites
Thinning	Selective sanitary felling	Reconstructive felling	Felling of the stands destroyed by forest pests and diseases
Severance felling ?			

Figure 3. Felling systems for the development, improvement and regeneration of forest stands.

of the stands. In commercial forests, the close-to-nature forest management should be aimed at ensuring high productivity, preserving the biotic stability of the stands and their habitat-forming functions. Felling and other activities are carried out with the least interference of machinery and mechanisms in the forest ecosystems. To maintain biodiversity, it is necessary to leave some old trees with a long lifespan, individual dead trees, and forest litter to mimic the processes in natural forests.

CONCLUSIONS

The research was conducted by means of experimental felling operations and other activities related to close-to-nature forestry; the most effective variants were identified, and the following conclusions were reached.

- Close-to-nature forestry includes a system of institutional and forestry activities aimed at restoring and developing resilient, multi-age, complex forest stands with a mixed composition both of natural and combined origin.
- In mature pine stands, after the first round of uniformly gradual felling with a decrease in density up to 0.3–0.4 (* ratio of the sum of tree crown projections to the occupied area) and measures to promote natural regeneration, within 4–5 years, the density of pine undergrowth was 8,000–20,000 trees·ha⁻¹ and there were additionally 2–9 thousand units ha⁻¹ of 1–2 year old self-seeding trees with uniform distribution on the plots. During the final cycle of uniformly gradual felling, the preservation of the undergrowth was 71–96%. At the age of 5–7 years, the mixed relatively uneven-aged pine young forest stands are developed.
- After stripped-coupe felling in pine stands of fresh and moist pine forests on sandy soils as well as after soil mineralization, the natural regeneration of Scots pine is satisfactory, and during 5–7 years the mixed young stands of pine are developed with the canopy closure of 0.3–0.4. (* ratio of the sum of tree crown projections to the occupied area).
- Conversion of artificial, even-aged and weakened pine stands of pure species composition into resilient mixed relatively uneven-aged forest stands is carried out by means of transformation logging operations with gradual removal of individual

trees and their groups to create proper conditions for the regeneration of high value species and development of the next forest generations.

- After carrying out the two cycles of transformation felling (complete course – 4 cycles) in the maturing artificial weakened pine stand of fresh oak-pine subor, repeated every 6 years, the mixed young pine stands of natural and combined origin have been developed after 11 years. In the gaps after the first cycle of transformation felling and implementation of restoration measures, the 11-year-old young mixed pine stands were formed with a share of Scots pine reserves from 2 to 9 trees in the composition. Somewhat worse regeneration was observed in the variants without any assisted regeneration (e.g. no sowing or planting), and provided that tending felling was not carried out in a timely manner. In 4–5 years after the second cycle of transformation felling, the regeneration performance was from 2,000 to 19,000 trees·ha⁻¹ of undergrowth and self-sown pine trees (the success rate of regeneration is estimated as good and satisfactory).
- To ensure the continuous forestry cover, it is advisable to use the final felling systems for the forest cover restoration, as well as felling systems aimed at improvement, regeneration and regeneration of the forest stands in the context of close-to-nature silviculture and sustainable forest management.

Author Contributions

AMZ: study conception and design, data collection and processing, models derivation, data analysis, writing of the manuscript (original draft). SK: data collection and processing, methodological discussions, writing of the manuscript (editing). IP: data collection, writing of the manuscript (editing). IK: writing of the manuscript (editing). TM: data collection and processing, checking and editing the references.

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Conflicts of Interest

The authors declare no conflict of interest.

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The Effects of Soil Type, Exposure and Elevation on Leaf Size and Shape in *Quercus cerris* L.

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ABSTRACT

One of the main environmental factors that influence plant species and community diversity are soil types, exposure and elevation. This study aimed to evaluate differences in leaf size and shape of *Quercus cerris* L. along environmental gradients in the Šumadija region in Serbia by using geometric morphometrics methods. The results showed significant differences between *Q. cerris* individuals inhabiting sites with different soil types, exposures and elevations. Individuals growing on nutrient deficient soils had smaller leaf size, elongated petiole, wide leaf blade, and higher values of fluctuating asymmetry compared to individuals growing on nutrient-rich soils whose leaf size was larger, more variable in shape and had lower values of fluctuating asymmetry. Additionally, individuals inhabiting higher elevations had elongated and narrow leaves and short petioles. Leaf size was also greater in individuals from lower elevations and north-exposed sites. The results of this study suggest that leaf morphological traits are affected by habitat differences and exhibit considerable plasticity in response to environmental demands.

Keywords: Turkey oak; habitat differences; leaf morphometrics; intraspecific variability

INTRODUCTION

The development of plant form and structure is regulated by genes and affected by the environment (Barkoulas et al. 2007, Fritz et al. 2018). Plants have the ability to display phenotypic plasticity to optimize resource utilization under different habitat conditions (Liu et al. 2020), and leaf morphology can represent one of the main determinants that reflect the status of the whole plant, thus being an excellent tool for ecological studies. Leaf traits vary across habitats with different climatic conditions (Olsen et al. 2013) and can provide insights into the evolutionary changes that enable plant adaptation to local environments.

In this study we investigated whether and to what degree leaf morphological traits of Turkey oak (*Quercus cerris* L.) are influenced by soil types, land exposure, and elevation. Oaks are suitable models for this kind of study, since they are represented by the large area of distribution and a broad ecological niche (Nixon 2006, Di Pietro et al. 2016, Jovanović et al. 2022a). Oaks are common, often dominant vegetation elements that include many ecologically diverse species which are widely distributed (Simeone et al. 2018).

The natural range of *Q. cerris* is southern Europe and Asia Minor. This species is characterized by good adaptability to different habitat conditions, it is tolerant to drought, air pollution, and it grows on a wide range of soil types. It can be found up to 1900 m above sea level, in hot climates on semi-shaded exposure, and in colder climates on sun-exposed sites (de Rigo et al. 2016). Although *Quercus* is one of the most commonly investigated genera regarding leaf morphology, studies of leaf morphological variability in *Q. cerris* are quite rare (e.g., Sisó et al. 2001, Čermák et al. 2008, Karavin 2014, Jovanović et al. 2022b).

One of the main environmental factors that influence the diversity of plant communities is soil type, as well as exposure and elevation gradients. Soil nutrients, for example, influence variation in plant morphology, including leaf functional traits (Su et al. 2021, Jovanović et al. 2022a). Litter leaves of plants growing on nutrient-rich soils are also rich in nutrients and maintain high soil fertility after decay (Gong et al. 2020). Previous studies have shown that increasing elevation, reducing precipitation and the amount of nutrients in the soil affect the reduction in leaf size (McDonald et al. 2003, Milla and Reich 2011), while the evaporative demand and

availability of water in the soil reflect different interactions of the morphology and the environment (Nicotra et al. 2011, Wang et al. 2019, Salehi et al. 2020, Kahveci 2023). Exposure also influences vegetation patterns (Yang et al. 2020) – at different exposures, differences occur in air and soil temperature, evaporation and transpiration, wind speed, and solar radiation (Bennie et al. 2008). In general, the polar-facing slopes are more humid and colder, with higher content of organic matter and deeper soil, while the equator-facing slopes are hotter and dryer, with lower levels of soil nutrients and more pronounced erosion. These conditions significantly influence vegetation through the modification of the local environment, causing differences in the morphological traits of the plants inhabiting such areas (Moeslund et al. 2013). Lastly, elevation also significantly influences vegetation distribution and attributes. Elevation gradients affect plant development and growth as they cause variations in environmental factors such as air temperature, solar irradiance, and rainfall (Liu et al. 2020). Additionally, plants are impacted by changing air pressure in different elevations – the reduction in air pressure at higher elevations lowers the partial pressure of the oxygen, decreasing the water vapour pressure and increasing the atmospheric transmissivity to solar radiation (Xu et al. 2021), further influencing photosynthetic parameters by affecting the trade-offs between these competing requirements. In habitats situated at greater elevations and on soils low in nutrients, plants show the tendency to decrease leaf area and increase leaf thickness (Liu et al. 2020). Although leaf traits can influence the fitness of trees on physiological, biochemical, morphological, and developmental levels (Donvan et al. 2011), intraspecific studies on how soil types, exposure and elevation affect leaf morphology in oaks (*Quercus* spp.) are scarce and mainly include physiological responses related to leaf nutrient composition changes in different environments (Li et al. 2006, Singh and Todaria 2012, Du et al. 2017, Azizi et al. 2020).

This study aimed to evaluate differences in leaf size and shape of *Q. cerris* along different soil types, elevations and exposures in the Šumadija region in Serbia. The leaves of *Q. cerris* vary in size and shape, are mostly oblong or oblong-elliptical, widest in the middle of the leaf blade, normally 9 to 12 cm long and 3 to 5 cm wide, with 7 to 9 pairs of triangular lobes (de Rigo et al. 2016). In the Šumadija region, *Q. cerris* is one of the edificatory species of the Hungarian oak and Turkey oak forests (*Quercetum frainetto-cerris* Rudski 1949), which are climatogenic forests typical for Serbia (Vukin and Rakonjac 2013, Jovanović et al. 2022b). The Šumadija region is characterized by high habitat diversity (Pavlović et al. 2017) – different soil types and elevation differences, along with significant areas under natural forest vegetation, make this site suitable for the investigation of the potential influence of habitat conditions on plant morphology. In the Šumadija region, small waterways dissected the relief, developing a hilly layout, with areas ranging from 100 to 1130 m above sea level. This region is characterized by high pedological diversity, with vertisol, cambisol and lithic leptosol being the most common soil types. Vertisol is formed in areas in which the change of wet and dry periods is well expressed, mostly on flat and slightly wavy relief, at 200–600 m of elevation, under natural vegetation of mixed deciduous

forests and grass communities (Čirić 1991). Cambisol (eutric) is the climatogenic type of soil of temperate-continental areas under the climatogenic plant community *Quercetum frainetto-cerris* Rudski 1949 (Brković 2015). It develops on a hilly relief on terrains with a lot of lime and shady sides where the water drains quickly (Pavlović et al. 2017). Skeletal soils (lithic leptosol) appear at the higher elevations of the studied area (300 m above sea level), where the relief is well developed. These soils are shallow, with rock fragments, common in areas where the parent rocks are subjected to continuous erosion. Thus, as leaf traits vary with the physical environment in predictable ways (Xu et al. 2021), the main objectives of this study were to evaluate leaf size and shape variation patterns between *Q. cerris* populations, and to link the observed patterns with habitat differences recorded for each population.

MATERIALS AND METHODS

Sampling

In the autumn of 2021, 138 randomly selected adult individuals of *Q. cerris* were sampled from nine natural populations in the central part of the Šumadija region in Serbia (Table 1, Figure 1). Šumadija, the central region of Serbia, occupies about 5800 km², covering the area between the Sava and the Danube in the north, Great Morava in the east, West Morava in the south, and Kolubara in the west (Šikanja 2019). *Q. cerris* leaves were sampled within Hungarian oak and Turkey oak forests (*Quercetum frainetto-cerris* Rudski 1949). To minimize clone selection risk, all sampled individuals from the selected populations were located at least 5 m from each other (Li et al. 2021, Jovanović et al. 2022c). From each individual, 10 fully developed leaves were collected (1380 in total), at the height of 8 to 10 m around the crown of each tree (Viscosi 2015), mainly under shaded conditions. Leaves were herbarized and scanned by placing an abaxial surface facing upwards on an Epson Stylus DX4050 scanner, with a resolution of 300 dpi.

Spatial Data

At each of the sampling sites, which were at least 10 km apart, latitude and longitude were recorded and used for obtaining information on soil types, exposure and elevation using QGIS 3.24.0 (QGIS Development Team 2022). The information on soil type was obtained from the imported digitalized maps using the data from Mrvić et al. (2013) and the Republic Geodetic Authority of Serbia (www.geosrbia.rs) which revealed three soil types at sampling sites – cambisol (eutric), vertisol, and lithic leptosol (Figure 2a). The information on the exposure and elevation (Figure 2b, c) was obtained by the SRTM Downloader Plugin in QGIS – Shuttle Radar Topography Mission (NASA Earth Data – <https://urs.earthdata.nasa.gov/>). This revealed six exposure groups – north, northeast, northwest, east, south and west, and nine elevation groups ranging from 288 to 444 m above sea level.

Landmark Configuration

On each leaf, 13 landmarks were recorded (Figure 3), following the methodology suggested by Viscosi (2015) using tpsDig and tpsUtil software (Rohlf 2015). The first three

Table 1. Sample size (N), soil type, exposure, elevation, latitude and longitude of the sampled populations of *Q. cerris*.

Population (Figure 1)	N	Soil type	Exposure	Elevation (m)	Latitude	Longitude
1	15	Vertisol	Northeast	288	44°7'30"	20°49'12"
2	13	Vertisol	West	222	44°14'18"	20°42'40"
3	18	Cambisol	Northeast	202	44°6'59"	20°56'43"
4	13	Cambisol	South	347	44°2'3"	21°0'42"
5	21	Vertisol	North	150	44°5'15"	20°58'49"
6	15	Vertisol	West	232	43°58'51"	20°54'1"
7	18	Cambisol	Northwest	272	43°55'44"	20°41'7"
8	18	Lithic leptosol	Northwest	373	43°55'2"	20°52'4"
9	7	Lithic leptosol	East	444	43°52'41"	20°56'53"

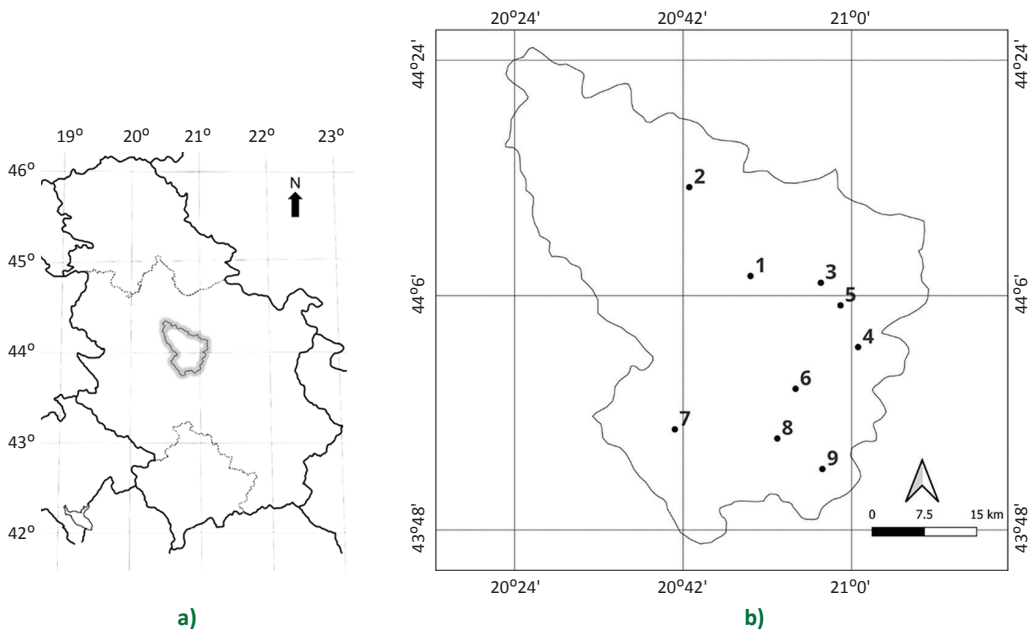


Figure 1. (a) the Šumadija region in Serbia, and (b) the enlarged sampling area showing sampling localities. Characteristics of each sampling locality (population) are presented in Table 1.

landmarks (landmarks 1-3) were unpaired and distributed along the midrib of the leaves, while the other landmarks (landmarks 4-13) were paired and distributed symmetrically on both sides of the leaves.

Statistical Analyses

Generalized Procrustes Analysis (GPA) was performed to minimize the sum of squared distances between the corresponding landmarks and to extract shape information by removing the information on size, location, and orientation (Savriama 2018). Procrustes ANOVA was performed to quantify leaf size and shape variation (Klingenberg 2003). In this analysis, centroid size (square root of the sum of the squared distances of all landmarks from their centroid)

was used as a measure of size (Rohlf and Slice 1990). The Canonical Variate Analysis (CVA) was performed to further visualize the differences between groups.

Fluctuating asymmetry (FA) was calculated by digitizing the leaf's left and right side separately and combining the two datasets into one. Procrustes ANOVA was performed on the combined dataset using individual and side as classifiers. When fluctuating asymmetry is present, the interaction of individual \times side is significant in the Procrustes ANOVA. The intensity of fluctuating asymmetry was measured by extracting the MS values from the interaction of individual \times side from the Procrustes ANOVA for each group (Benítez et al. 2020). All statistical analyses were performed in MorphoJ software (Klingenberg 2011).

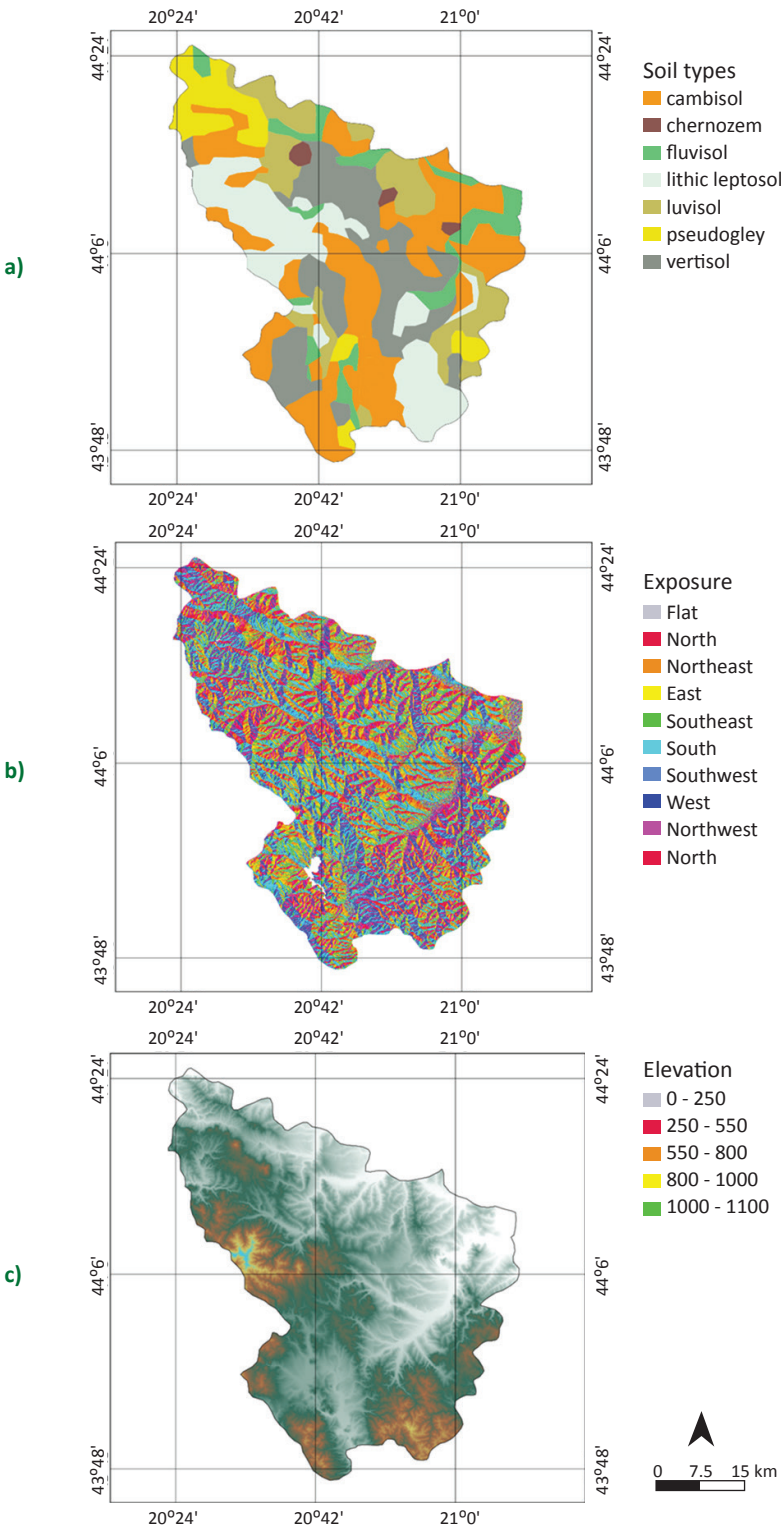


Figure 2. Spatial data of the study region: (a) soil map, (b) exposure map, and (c) elevation map.



Figure 3. Configuration of *Q. cerris* leaves showing 13 landmarks: 1) beginning of the petiole, 2) junction of the blade and the petiole, 3) apex of the leaf blade, 4) and 9) base of the apical sinuses of the blade tip (right and left side), 5) and 10) tip of the lobe immediately beneath the apex of the leaf blade (right and left side), 6) and 11) tip of the lobe at the largest width of the blade (right and left side), 7) and 12) base of the sinus immediately beneath the lobe of the landmarks 6) and 11), 8) and 13) the first basal lobe of the blade (right and left side).

RESULTS

Procrustes ANOVA of leaf size and shape indicated that both size and shape show significant differences between individuals growing on different soil types, expositions and elevations (Table 2). Additionally, ANOVA of the centroid size indicated a statistically significant effect of soil type

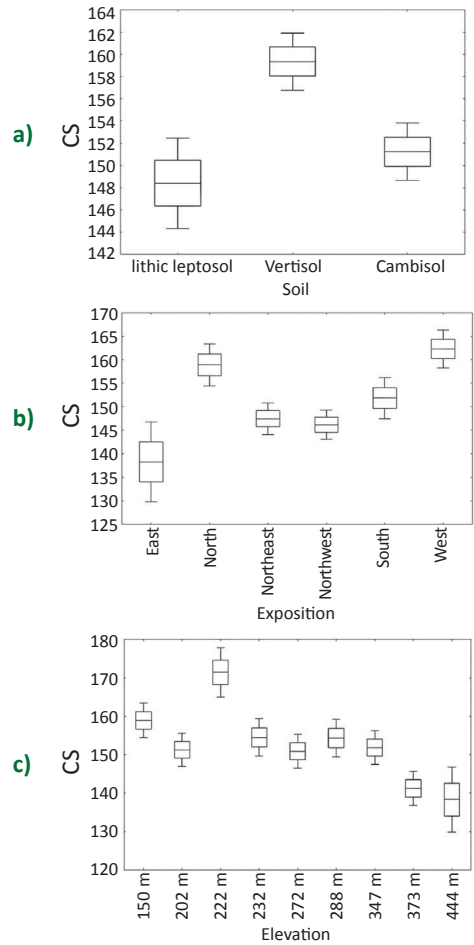


Figure 4. Means, standard errors and 95% confidence intervals of leaf centroid size (CS) of *Q. cerris* individuals growing on: (a) different soil types, (b) expositions, and (c) elevations.

($F=23.11$; $P<0.01$), exposition ($F=12.74$; $P<0.01$) and elevation ($F=14.24$; $P<0.01$) on leaf size (Figure 4). Individuals growing on lithic leptosol, on east exposition, and at higher elevations, had lower values of centroid size compared to individuals growing on cambisol and vertisol, north and west expositions and at lower altitudes, which had higher values of centroid size.

Table 2. Results of the Procrustes ANOVA of *Q. cerris* leaves of individuals growing on different soil types, expositions and elevations.

		SS	MS	df	F	P
Soil type	Shape	0.622	0.014	44	14.40	<0.01
	Size	67803021.26	3390160.63	2	24.99	<0.01
Exposition	Shape	1.42	0.01	110	13.51	<0.01
	Size	9226782.09	1845356.42	5	13.76	<0.01
Elevation	Shape	2.19	0.01	176	13.37	<0.01
	Size	15550775.03	1943846.88	8	14.98	<0.01

Canonical variate analysis indicated considerable overlapping between individuals growing on different soil types (Figure 5). However, some grouping patterns were observed – individuals growing on vertisol had narrower lower part of the leaf blade, higher lobation and shorter petiole compared to individuals growing on cambisol and lithic leptosol.

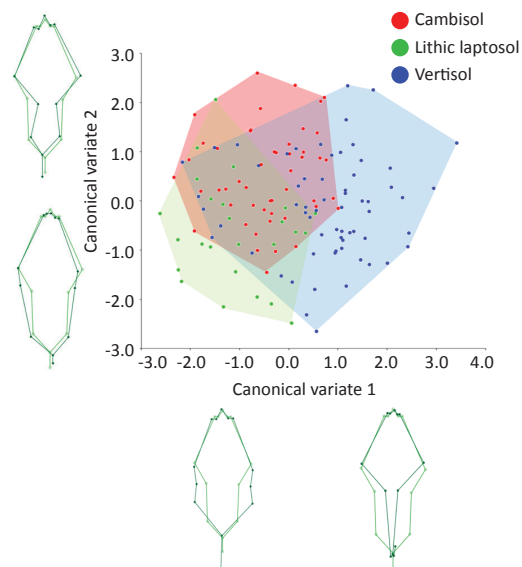


Figure 5. Ordination of *Q. cerris* individuals growing on different soil types within the first two canonical variates obtained by the canonical variate analyses. Shape changes along different soil types are represented by wireframe charts.

Individuals growing on lithic leptosol had rounder leaf blades, with lesser lobation, elongated petiole and a wide leaf blade. Individuals growing on cambisol had a wide upper part of the leaf blade and an elongated petiole. Canonical variate analysis also showed some grouping patterns in regard to exposition (Figure 6). Individuals growing on the east-exposed sites differed from others by having narrow leaf blades and elongated petioles. Individuals growing on west-exposed sites had shorter petioles and a narrow lower part of the leaf blade. Individuals growing on north, northeast and northwest-exposed sites grouped based on a long petiole and a wide leaf blade. Individuals growing on south-exposed sites had a wide upper part of the leaf blade and a short petiole. For different elevations, despite the considerable overlap, some grouping patterns were also observed (Figure 7). In general, individuals from higher elevations had elongated and narrow leaves and short petioles, compared to individuals growing on lower altitudes which had shorter and wider leaf blades and elongated petioles.

Procrustes ANOVA for leaf side of each individual (interaction individual \times side) showed significant differences ($F=3.25$; $P<0.01$), indicating the presence of fluctuating asymmetry. The highest levels of fluctuating asymmetry were recorded in individuals growing on lithic leptosol, north-exposed sites and at lowest altitudes (Figure 8).

DISCUSSION

The results of this study showed significant differences in leaf size and shape between *Q. cerris* individuals inhabiting sites with different soil types, exposures, and elevations, suggesting that leaf morphological traits are

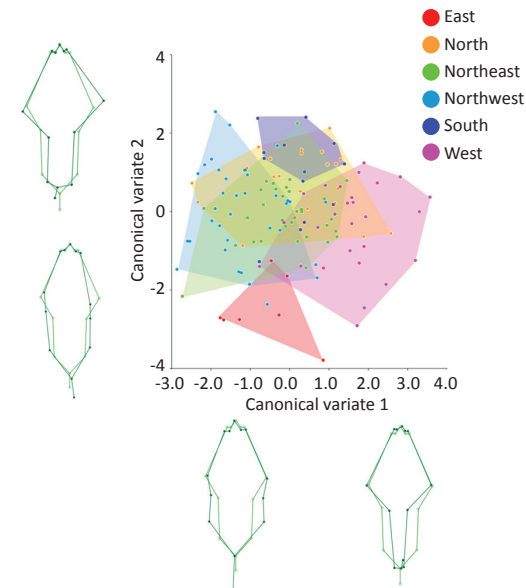


Figure 6. Ordination of *Q. cerris* individuals growing on different expositions within the first two canonical variates obtained by the canonical variate analyses. Shape changes along different exposures are represented by wireframe charts.

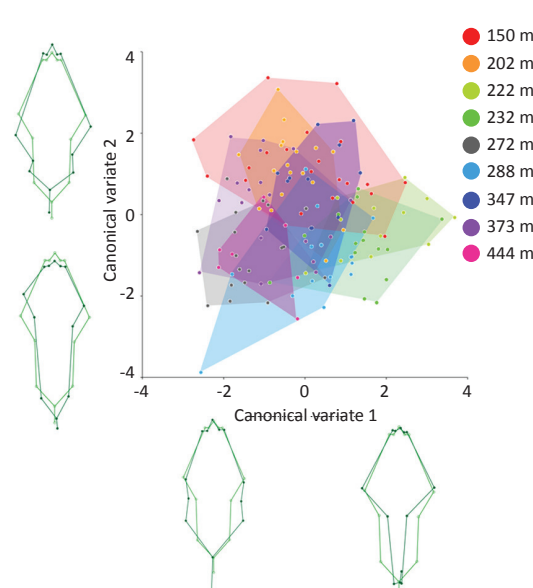


Figure 7. Ordination of *Q. cerris* individuals growing on different elevations within the first two canonical variates obtained by the canonical variate analyses. Shape changes along different elevations are represented by wireframe charts.

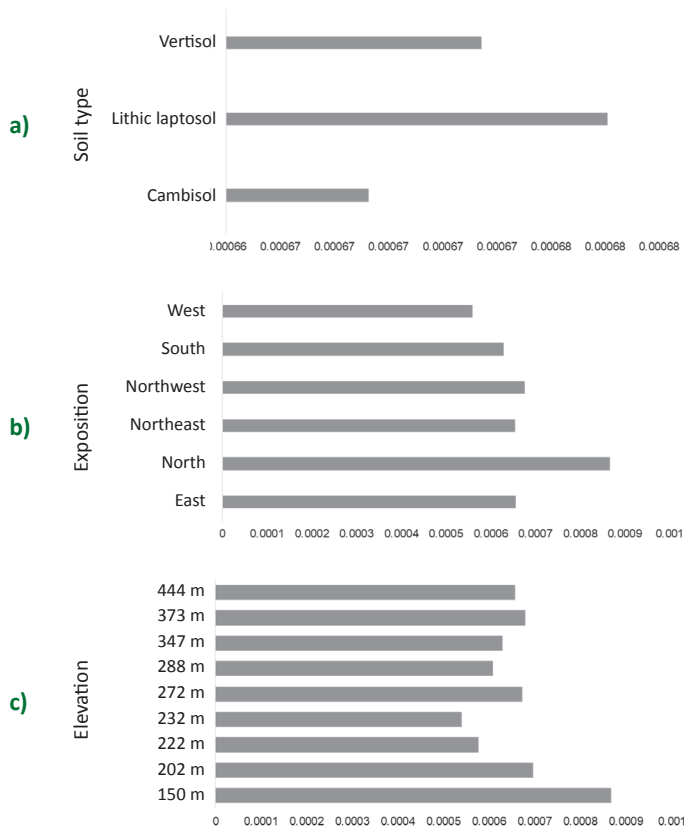


Figure 8. Values of fluctuating asymmetry of *Q. cerris* individuals growing on: (a) different soil types, (b) expositions, and (c) elevations.

affected by habitat differences and exhibit considerable plasticity in response to environmental demands. Leaves of individuals growing on nutrient-rich soils (cambisol and vertisol) were larger compared to the leaves of individuals growing on skeletal soils (lithic leptosol). Individuals from lithic leptosol also differed from others by more round leaf blade, less pronounced lobation, elongated petiole and a wide leaf blade. Additionally, the leaves of individuals from higher elevations were smaller compared to the ones growing at lower elevations, with elongated and narrow leaves and a short petiole. Individuals growing on northern exposures had larger leaf sizes, elongated petioles, and wide leaf blades, contrary to the individuals growing on southern exposures, which had smaller leaves and shorter petioles.

In the Šumadija region lithic leptosols are relatively variable in physical and chemical properties and are usually poor in nutrients, and have poor water regime (Veljović 1967, Pavlović et al. 2017, Jakšić et al. 2021), while cambisol and vertisol are moderately rich in nutrients (N, P, K) and have more favourable water regimes. This high soil diversity in the Šumadija region in Serbia conditioned the existence of different productivity levels – productivity of deep soils is considered to be higher compared to shallow skeletal soils (Ličina et al. 2011, Jovanović et al. 2022b). Thus, cambisol

and vertisol (nutrient-rich soils with more favourable water regimes) present at lower elevations provide suitable conditions for *Q. cerris* to develop larger leaves. Moreover, leaves of shaded plants tend to be slightly larger than those of full-sun plants (Stanton et al. 2010), as larger leaf areas receive light energy for photosynthesis at sites where light levels are low, explaining larger leaves at the lower elevations of the study area. When observing the connection between leaf traits and expositions, north-exposed sites have a higher content of soil nutrients and are moist, which influences leaf size increase (Moeslund et al. 2013), as suggested by the larger leaves, with a broad leaf blade and an elongated petiole at the northern expositions of the study region.

Environmental factors, including air temperature, radiation, and soil nutrients vary with elevation – temperature decreases, and precipitation and radiation increase with higher altitudes (Guo et al. 2018). Soil nutrients also change at different elevations – soil organic carbon (C) concentration may increase, while the availability of soil nutrients, such as N and P, may decrease with increasing elevation (He et al. 2016). Smaller leaf sizes are favoured at higher altitudes, characterized by higher insolation and higher light-capturing surface built by the plant per unit investment of dry mass, optimized to maintain

a positive carbon balance and influence the fitness of the whole plant (Pan et al., 2013). Additionally, larger leaves in northern exposures shed heat more slowly and are heated above air temperature more compared to smaller leaves. Transpiration is also effective in shedding heat – when there is less total foliage per unit ground area, more leaves are exposed to direct radiation (McDonald et al. 2003). In general, smaller leaves are advantageous at high intensities of solar radiation, while larger leaves which have less efficient energy exchange capacity are advantageous in habitats with lower irradiance (Wang et al. 2019). The differences between leaf traits along different habitats in this study indicate that in *Q. cerris* morphology can reflect environmental demands, although it must be noted that other factors, such as genes and development, also play important roles in the observed leaf variability.

Fluctuating asymmetry values were the highest in individuals growing on nutrient-poor soils, in both high and low altitudes, and in north-exposed sites. The differences in fluctuating asymmetry between traits are explained by varying levels of developmental stability, which can be related to trait functionality, selection mode, and stress associated with the development processes (Aparicio and Bonal 2002). Fluctuating asymmetry is a reliable measure of plant stress at local scales and it can be used as a biological tool for monitoring environmental quality (Cornelissen and Stiling 2010). This small and random deviation from bilateral symmetry (Cornelissen et al. 2003), used as an indicator of developmental instability, is more pronounced if the plant's adaptive mechanism fails to buffer stress (Graham et al. 2010). In this study, higher values of fluctuating asymmetry recorded in nutrient-deficient, shallow soils (lithic leptosol), suggest that the ability of *Q. cerris* to buffer environmental stress is reduced, causing deviations between the left and the right side of the leaf. In the Šumadija region, lithic leptosol is present at higher altitudes where stress levels are also higher compared to the lower elevations. High values of fluctuating asymmetry were also recorded in north-exposed sites, which are characterized by decreased insolation, lower temperature, and higher precipitation. Thus, the connection between high values of fluctuating asymmetry and more severe environmental conditions is not as straightforward, suggesting that other phenomena, such as phenotypic plasticity, can be more sensitive to stress than fluctuating asymmetry (Graham et al. 2010). A plastic response regarding leaf morphology to varying environments has been found to be higher in many *Quercus* species, enabling relatively quick adaptation to different environmental conditions (Blue and Jensen 1988, Ashton and Berlyn 1994, Kusi 2013). Plastic responses enable *Quercus* species to cope with adverse environmental conditions by modifying leaf morphological traits, such as water transport, heat reduction, prevention of photochemical damage, and the preservation of minimum photosynthetic rate (Dickson and Tomlinson 1996).

Although the results of this study showed differences in leaf size and shape between *Q. cerris* individuals growing at different environmental gradients, leaf trait relationship with environmental conditions showed considerable over-

lapping, suggesting that within-site variation may also be the source of the observed variability (Gong and Gao 2019). Plant and climate relationships at the intraspecific level are influenced by ecotypic variation of plant traits and their plasticity (Royer et al. 2008), and these relationships can be used as indicators of the levels of environmental stress present in a certain area. Thus, understanding how plants adapt to different habitat conditions is important in conservation strategies, especially when dealing with species that have large areas of distribution and a broad ecological niche (Šijačić-Nikolić et al. 2021). In such cases, leaf morphology can be used as a reliable indicator of habitat quality (Xu et al. 2021).

CONCLUSIONS

This study showed that leaf morphology is related to environmental conditions of *Q. cerris* from the Šumadija region in Serbia, as geometric morphometrics analysis revealed differentiation of *Q. cerris* individuals growing on different soil types, expositions and elevations. The observed differentiation was determined by both leaf size and shape. However, the results indicated the environmental impact on the variation patterns of the leaf in *Q. cerris* on a relatively small spatial scale. Future studies should include a larger sample of different oak species from diverse areas across a broader area of distribution to better understand how habitat influences leaf morphology. Along with the environmental influences, the variability of leaf morphology can also be attributed to genetic factors, making molecular analyses another priority in future research.

Author Contributions

MJ and JM conceived and designed the research, MJ carried out the field measurements, MJ and FG processed the data and performed the statistical analysis, MŠN, MN and IKJ supervised the research and helped to draft the manuscript, MJ and FG wrote the manuscript, and all authors provided comments.

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Conflicts of Interest

The authors declare no conflict of interest.

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Diameter-Height Growth Performance of Natural Species of Central Anatolian Forest Steppe in Terms of Influencing Site Conditions

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ABSTRACT

Trees' height (H) and diameter (D) growth depend on many factors and vary between species. This study examined H and D growth of *Juniperus excelsa*, *J. foetidissima*, *Pinus nigra*, *Quercus cerris* and *Q. pubescens*, growing naturally in the Central Anatolian forest steppe and the site conditions (human impact, woody plant coverage, tree density, altitude, exposure) that influence H and D growth. The present study hypothesises that the decline of height growth might indicate limited rainfall in the region. Two datasets were distinguished for the statistical analysis: the first comprised maximum height (MH) and diameter (MD), human impact, woody plant coverage, and tree density of the sampling plots, and the second comprised all measured Hs and Ds of the sampling plots, exposition, and altitude. Variance and correlation analysis were applied to both datasets to determine the relationships between parameters. Non-linear regression analysis was applied to both datasets to provide H-prediction equations. According to the results of statistical analyses applied to two datasets, each tree species reacted differently to the site conditions. However, the most relevant relationship was found between height and diameter growth for all species. The MH-MD and D-H of *P. nigra* (except the altitude) and *Quercus cerris* + *Q. pubescens* (except the human impact) did not respond to any of the site conditions remarkably, while those of *J. foetidissima* responded to all of the site conditions examined. The H and D of each species were affected by the exposure. While the highest number of trees was found on N-exposed slopes, the heights trees of each species were found on N- and NW-exposed slopes. The results of non-linear regression analysis applied on both datasets of H-prediction equations of each species involved different parameters, even though the diameter was the only relevant variable for height prediction. Although it is not possible to reach a definite conclusion for other species within the scope of this study, *P. nigra* had a shorter height in Central Anatolia than in areas with better environmental conditions. Height growth might indicate water limitations of Central Anatolian region, but genetic code might be an important factor of how a species will cope with drought.

Keywords: drought; variation of maximum height and diameter; height decline; human impact; height prediction

INTRODUCTION

The relationship between diameter (D) and height (H) in trees provides fundamental information for forest research (Sumida 2015). The H-D relationship changes with environmental conditions and over time (Sumida et al. 2013). Indeed, each tree may follow a different path in terms of growth (King 2011). Moreover, depending on local conditions, this relationship can vary between locations and regions (Fulton 1998). Central Anatolia as an arid region and it can have disadvantageous characteristics for tree growth due to

droughts, which can also reduce forest productivity by changes in photosynthesis rate (Grassi and Magnani 2005), carbon assimilation (Lawlor and Cornic 2002), phenology (Misson et al. 2011), and tree morphology (Aspelmeier and Leuschner 2006). These characteristics have adverse impact on ecosystem stability, can determine the existence of species within a region, and have a wider distribution related to geographical conditions (Chambers et al. 1999). Moreover, various species living in the same region may show diverse growth characteristics (Bond et al. 2000), as distinct plant groups can apply different resource-use strategies (Lyon and Sagers 1998).

Plant growth depends on photosynthesis, stored reserves of non-structural carbohydrates, and other physiological functions (Chapin et al. 1990). The mechanism of radial growth is affected by five factors: water, carbon, nutrients and temperature, auxin (a plant hormone involved in the cambial activity), and mechanical stress (Zweifel et al. 2006). Radial growth depends primarily on the current tree water levels and secondarily on carbon balance. However, different mechanisms are involved in height growth. Trees are thought to have a mechanism that slows their growth with increasing age because the relative top heights of the young stands are significantly higher than those of the older stands (Gulyás et al. 2019). Several mechanisms have been proposed to explain this phenomenon and the differences in H growth and maximum H within species, including reduced respiration rate, limitations of nutrient availability, maturational changes, and hydraulic limitations (Ryan and Yader 1997). Furthermore, genetically programmed slowing of growth may explain the decrease in H growth with age (Greenwood 1989).

Central Anatolia is a semi-arid region with low precipitation and high evaporation rates, unfavourable natural environmental conditions, and long-term history of human activity and land degradation resulting in a significant decrease in the number of trees and reduced woody vegetation cover (Kahveci 2017). Although it may seem difficult to distinguish the extent to which these phenomena have been caused by human and natural influences, it will become easier to reach clear conclusions as the number of research studies increases. Some native tree species have survived under the adverse conditions of Central Anatolia: *Pinus nigra* J. F. Arnold (Austrian pine), *Quercus cerris* L. (Turkey oak) and *Q. pubescens* Willd. (white oak); *Juniperus excelsa* M. Bieb (Crimean juniper); and *J. foetidissima* Willd (Foedit odour juniper). These are generally distributed in relict areas of the mountain forest steppes (Makunina 2016, Kahveci 2017). These trees also occupy many other environments. However, tree growth varies among regions because of site conditions (Bariboult et al. 2012).

This study examined the H and D growth of natural tree species and the influence of the site conditions (human impact, woody plant coverage, tree density, altitude, exposure) on H and D growth in the Central Anatolian forest steppe. The aims of this study were: (i) to determine the variation of both maximum height (MH) – maximum diameter (MD) and H-D growth between natural tree species of Central Anatolia, (ii) to ascertain the extent to which site conditions influence both MH - MD and H-D growth, and (iii) to develop height prediction equations in terms of affecting site conditions. Even though the limiting factor (light, soil properties, water, ext.) of tree growth varies between forest ecosystems (Gao et al. 2018), water-stressed environments, especially severe drought, can cause the growth decline of trees (O'Brien et al. 2017). Therefore, it is expected that the species that spread over large areas have shorter tree heights in the semi-arid conditions of Central Anatolia.

MATERIALS AND METHODS

Research Site

The present study was carried out in the following six provinces (including 16 locations) of Central Anatolia: Ankara (Elmadag, Bala, and Nallihan), Eskişehir (Mihalıççık, Alpu, Seyitgazi, and Çifteler), Kırıkkale (Bahşılı, Yahşihan, and Delice), Çorum (Sungurlu, Boğazkale, and Uğurludağ), Yozgat (Merkez), and Konya (Merkez, and Ilgın) (Figure 1, Table 2). The most common lithological units at the research site were limestones: crystallised, lacustrine, neritic, and andesite (Balkan 2017). Leptosols, calcisols, and cambisols were the dominant soil types according to the soil classification system of the World Reference Base for Soil Resources (Dinc et al. 1997).

Based on the Köppen–Geiger climate classification, the research site had type Bsk (cold semi-arid) and type Dsa or Dsb (continental with either hot or cold dry seasons) climates (Köppen et al. 1954, Öztürk et al. 2017). Meteorological data



Figure 1. Research site in Central Anatolia. Locations: 1- Mihalıççık, 2- Alpu, 3- Seyitgazi, 4- Çifteler, 5- Nallihan, 6- Elmadag, 7- Bala, 8- Bahşılı, 9- Yahşihani, 10- Delice, 11- Sungurlu, 12- Boğazkale, 13- Yozgat, 14- Uğurludağ, 15- Konya, and 16- Ilgın.

for the research site (monthly precipitation, temperature, and humidity) were obtained from the General Directorate of Meteorology (GDM) for the period from 1927 to 2020 (GDM 2020). The average annual total rainfall varied between 329.2 mm in Konya and 570.3 mm in Yozgat (Table 2). The highest average annual temperature was 12.45°C in Kırıkkale. The lowest average annual humidity was 60% in Konya and Ankara (Table 1). The average length of the growing season was 160 days at the research site (Atalay and Gökçe Gündüzoğlu 2015).

Humans have used forest resources for thousands of years. Oaks and junipers have been used as fuel and animal feed. Unlimited grazing, which is still ongoing, continues to degrade both forest areas and grasslands. These woody species survived despite these effects: *J. foetidissima*, *J. excelsa*, *J. oxycedrus* L., *Pinus nigra*, *Quercus cerris* L., *Q. pubescens* Willd., *Amygdalus communis* L., *Berberis crataegina* DC., *Crataegus orientalis* Pall. ex M.Bieb., *C. monogyna* Lacq., *Cotoneaster nummularius* Fisch. & C.A.Mey., *Colutea cilicica* Boiss. & Balansa, *Cistus laurifolius* L., *Jasminum fruticans* L., *Lonicera* sp., *Paliurus spina-christi* Mill., *Cornus sanguinea* L. subsp. *Australis*, (C.A.Mey.) Jáv., *Origanum minutiflorum* O.Schwarz & P.H. Davis., *Pistacia palaestina* Boiss., *Pyrus elaeagnifolia* Pall. subsp. *elaeagnifolia*, *Prunus domestica* L., *Rhamnus rhodopea* Velen., *Rosa canina* L., *R. pulverulenta* M. Bieb., *R. thymifolia* Bornm., *Spiraea crenata* L., and *Ulmus minor* Mill.

Sampling Methodology

The forest management plans of eight provinces were used to determine suitable locations with forest relicts. When selecting sampling plots in these locations, the primary motivation was the occurrence of groups of trees that were in relatively good condition. For this reason, plots were not chosen according to a specific method. Since it was not possible to find excellent and pure stands of each species in the same location, sample plots were taken from different locations (Table 2). Although all of these stands were located in the Central Anatolian region, climatic conditions vary between locations (Table 1). Rectangular plots (25 × 20 m) were used for recording: D at a breast H of 1.37 m (DBH), in cases with DBH > 5 cm and Hs in each plot; longitude and latitude (Universal Transverse Mercator [UTM]); Alt; Exposure (°); slope (%); the number of trees; human impact rated as 0 = no impact, 1 = ancient and early medieval impact, 2 = tree cutting, 3 = commercial use, fire,

or beekeeping; 4 = destruction of the forest; 5 = complete destruction of the forest (Samojlik et al. 2013); woody plant coverage according to the cover abundance scale (Domin 1928) rated as + = single tree, 1 = one to two trees, 2 = <1% cover, 3 = 1–4% cover, 4 = 4–10% cover, 5 = 11–25% cover, 6 = 26–33% cover, 7 = 33–51% cover, 8 = 52–75% cover, 9 = 76–90% cover, and 10 = 91–100% cover by woody plants. In total, 148 plots were sampled from 2005 to 2020 (Table 2).

Data Analysis

Statistical analysis was carried out in R (RStudio 2020). Variance analysis and Pearson correlation analysis were carried out on various versions of datasets, but only the results two datasets were statistically significant. Dataset 1 was prepared based on plots and it included: MD and MH of plots of five tree species (n = 143; *P. nigra* = 37; *Q. cerris* + *Q. pubescens* = 30; *J. excelsa* = 45; and *J. foetidissima* = 31), altitude, human impact, tree density (TD = number of trees per plot*10,000/500) and woody plant coverage. MH and MD of trees were used for analysis because maximum values may be the distinguishing features of species in the locations. On the other hand, it was previously stated that *Q. cerris* and *Q. pubescens* species grow naturally in the research area. Since *Q. pubescens* was dominant in the regions and had about 80% of individuals in the plots, and *Q. cerris* did not contain a scientifically acceptable number of individuals, both species were combined as oak (Quer) for the analysis. Dataset 2 comprised all D and H values of each species (n = 1,397; *P. nigra* = 441; *Q. cerris* + *Q. pubescens* = 331; *J. excelsa* = 417 and *J. foetidissima* = 208) measured in 143 sampling plots on the species basis, altitude, and exposure. Variable exposure only correlated with all measured H and D values. Height prediction equations were provided for both datasets by using non-linear regression analysis.

RESULTS AND DISCUSSION

Results of variance analyses applied on two datasets for comparing the species (*J. foetidissima*, *J. excelsa*, and *P. nigra*, *Q. cerris* + *Q. pubescens*) showed that all of the variables were statistically distinct from each other, and MD (p<0.001), MH (p<0.001), tree density (p<0.001), woody plant coverage (p<0.05), human impact (p<0.05), D (p<0.001) and H (p<0.001) were statistically significant. The results of the variance analyses were visualised in boxplots

Table 1. Long-term (1927–2020) average climate data based on observations from meteorological stations in the research site.

Station	Observation Period (Year)	Average annual total rainfall (mm)	Average annual temperature (°C)	Average annual humidity (%)
Ankara	93	393.2	11.9	60.0
Eskişehir	92	372.8	11.3	65.2
Kırıkkale	57	388.0	12.5	61.8
Konya	91	329.2	11.7	60.0
Çorum	91	430.7	10.8	64.6
Yozgat	91	570.3	9.2	66.8

Table 2. Main characteristics of the research site.

Location	Coordinates (N, E)	Altitude (m)	Species	Tree Density	Number of Plots
1-Mihaliççık *	39°51'18.9", 31°30'34.2"	1077-1128	Jufo	40-280	8
2-Alpu *	39°54'50.2", 31°09'23.9"	1235-1286	Jufo, Juex	200-540	9
3-Seyitgazi *	39°22'35.7", 31°02'20.1"	1047-1202	Jufo, Juex	80-560	15
4-Çifteler*	39°11'41.8", 30°43'33.1"	1359-1400	Jufo	100-420	8
5-Nallıhan*	39°45'39.5", 30°57'58.3"	677- 946	Juex	120-260	4
6-Elmadag*	39°47'29.8", 33°15'49.6"	970-1099	Juex	100-240	12
7-Bala*	39°47'29.8", 33°15'49.6"	1239	Pini	400-520	2
8-Bahşili**	39°43'42.1", 33°21'21.2"	980-1200	Juex	100-260	10
9-Yahşihan**	39°47'37.8", 33°21'05.1"	1010-1030	Juex	100-220	10
10- Delice**	39°56'57.1", 34°01'50.8"	1150	Quer	400-430	2
11-Sungurlu***	40°09'54.7", 34°22'35.0"	1085-1500	Pini, Quer	140-580	6
12-Boğazkale***	40°01'16.3", 34°36'50.4"	1200-1518	Pini, Quer	80-480	16
14-Uğurludag***	40°26'45.6", 34°27'20.1"	1003-1260	Pini, Quer	200-500	40
13-Yozgat	39°48'18.7", 34°49'30.1"	1550	Pini	180-200	2
15-Konya	39°47'29.8", 33°15'49.6"	1390	Pini	100-320	2
16-Ilgın (Konya)	38°16'17.7", 31°54'54.6"	1680	Pini	400-820	2
Total					148

* Province Eskişehir, **P. Ankara, *** P. Kırıkkale, **** P. Çorum, Pini = *Pinus nigra*, Jufo = *J. foetidissima*, Juex = *J. excelsa*, Quer = *Q. cerris* + *Q. pubescens*.

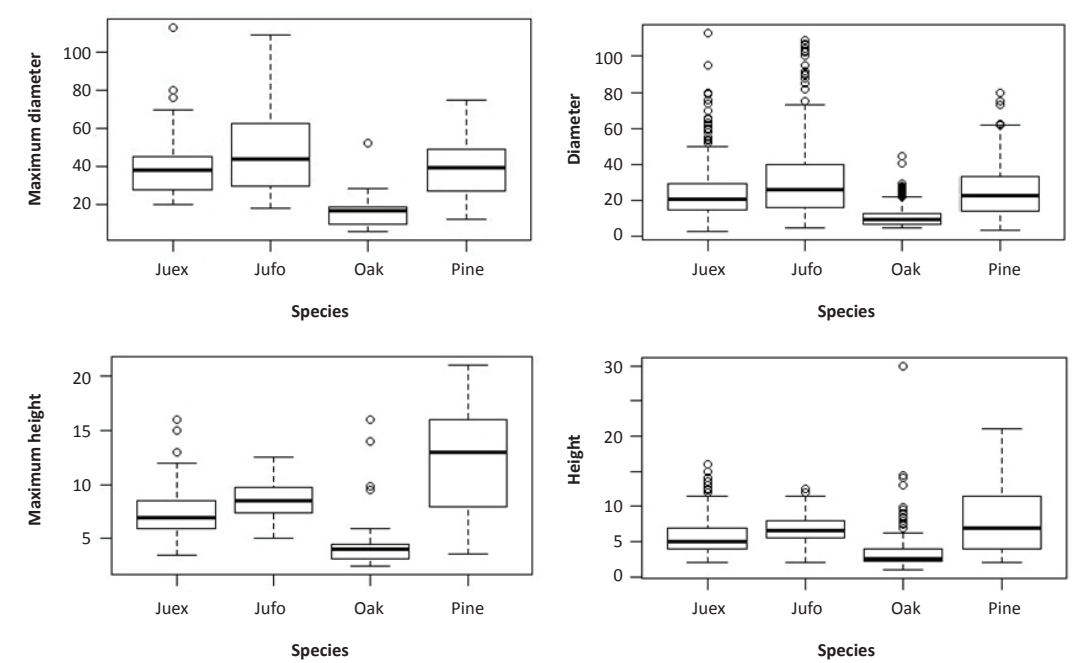


Figure 2. Comparison of maximum height (MH) – maximum diameter (MD) and height (H) – diameter (D) four tree groups visualised by boxplots.

showing the minimum, maximum, and median values of the measured parameters for the tree species (Figure 2, Figure 3, and Figure 5).

Comparison of Both MH – MD and H – D Growth

Tree growth varies with temperature and water availability. At global scales, tree height peaks in regions with mild mean annual temperatures (around 12–16°C), low water stress, and low seasonality (Larjavaara 2014). Each tree can survive between certain altitudes, but still, there is an optimal altitude at which trees grow best and build more stable stands. The forest relicts in the Central Anatolian region might have remained at this optimal altitude, where the tree species can reach maximum height and diameter (Kahveci 2021). This optimal altitude varies between species. The plots related to *P. nigra* were located at the altitude between 1,100 and 1,680 m above sea level (a.s.l.), whereas the plots related to *J. foetidissima*, *J. excelsa*, and *Q. cerris* and *Q. pubescens* were located at an altitude between 677 and 1,400 m a.s.l. (Table 2).

According to the results of variance analysis applied to both datasets, *J. excelsa* and *J. foetidissima* comprised a group concerning MH-H, while *J. excelsa* and *P. nigra* comprised another group concerning MD-D. By comparing the box plot of maximum diameter and all diameters of each species no significant differences between the boxplots were found. However, there are slight differences between the box plots of the maximum height and all measured heights. Nevertheless, many factors such as climatic conditions, environmental factors (altitude, location slope, slope direction, and human activity) (Jiao 2017), biological characteristics, and tree physiology (Zweifel et al. 2006) play an important role in tree growth. However, the genetic code also controls it (Martin-Benito et al. 2008). The genetic code might decide how much a tree is affected by the environmental conditions in which it grows, since natural species of Central Anatolia show different growth characteristics.

Oak is the most widespread tree species in Central Anatolia (Davis 1971, Hedge and Yaltrık 1982). The genus *Quercus* has spread throughout the northern hemisphere in Asia, North America, Europe, and Africa (Axelrod 1983), down to the Equator, and comprises approximately 400–500 species (Nixon 1993, Valencia et al. 2016). However, it is challenging to find oak stands in good condition in Central Anatolia because of thousands of years of past exploitation. Modern forest management authorities have also been exploiting oak through coppicing with 20-year intervals for many years. Therefore, only individual older oak trees were found in the region. The oaks had regenerated through stump sprouting, which has an advantage over seedlings since ample carbohydrates are available from the parent stool and its root system, so new shoots thrive from the start (Hölscher et al. 2001). This can be seen as an advantage in H growth; however, growth and health decline can be experienced during coppice management (Desprez-Loustau et al. 2006). In this context, values of 14 m MH and 22 cm MD were measured at the research site. These values were relatively low compared to the same species in other regions. Berta et al. (2019) measured an MH of 22.30 m and an MD of 44.35 cm for *Q. pubescens* on the Croatian Adriatic coast.

P. nigra is a long-living tree growing across the northern part of the Mediterranean basin, from Spain to Turkey (Isajev et al. 2014). *P. nigra* has the ability to grow on poor and bare soils, is used for ecological restoration, and generally forms stands with trees belonging to the *Quercus* genus (Marchi et al. 2018). Although *P. nigra* is Turkey's second most common coniferous tree spreading, it shows a limited distribution in the Central Anatolian Mountains. It forms both pure and mixed stands with oak. According to the results of this study, *P. nigra* had an MH of 22 m in the best growing environment in Yozgat (Figure 2), which was not representative of the research site because the average rainfall (570 mm) in Yozgat was much higher than on other locations (Table 1). Another issue was that *P. nigra* had wide varieties in Anatolia, and one of the varieties with three-needle leaves was found in Yozgat (Saatçioğlu 1955). Although this is the subject of another study, *P. nigra*'s genetic characteristics might enable it to adapt to adverse conditions and may create varieties to increase its adaptability to changed climatic conditions. The average H measured for the *P. nigra* area was 8 m in the research site. Raptis et al. (2021a) researched H–D relationships in the Olympus National Park, Greece, which had relatively better climatic conditions, and measured an MH of 30.40 m and an MD of 85.40 cm. Raptis et al. (2021b) measured an MH of 29.80 m and an MD of 74.60 cm in the Troodos National Forest Park in Cyprus. Özçelik et al. (2013) measured H and D in nine locations dominated by the Mediterranean climate, where the annual precipitation varied between 882 and 1,351 mm. The results were as follows: MH 24.60–30.40 m, MD 61–86 cm and mean H between 17.09 and 20.72 m. There was a significant difference between these data and the data obtained from the research site.

Genus *Juniperus* is the second most diverse genus of conifers, with 67 species worldwide, and is mainly confined to the northern hemisphere (Adams 2004), as are *Quercus* spp. The species show a wide range of climatic plasticity, colonising sites that vary from sub-humid to the semi-arid steppe zone of the Mediterranean region (Quézel and Médail 2003). *J. excelsa* is a monoecious tree that occurs in south-eastern Europe and south-western Asia and can reach an H of 20–25 m (Boratyński et al. 1992). It is frequently seen in the arid regions of Anatolia, tolerates severe drought and cold conditions, and can grow on shallow and degraded soils (Akman 1979). However, *J. excelsa* stands have been degraded in Central Anatolia. The MH of *J. excelsa* was measured as 13 m (with an average tree density of 200) in the research area, which can be described as relatively short. However, Stampoulidis and Milios (2010) found MH values of 12 m and 14 m in medium- and good-quality stands, with tree density ranging from 80 to 580 in Prespa National Park in Greece, which had 817 mm rainfall. Douaihy et al. (2011) found MD values of 8–9 m with a tree density of 257 trees·ha⁻¹ in Lebanon, where there was 600 mm precipitation.

J. foetidissima grows in the southeast and east Europe, Caucasus, and West and Central Asia, mainly above shallow rocky soils (Farjon 2021). Adult *J. foetidissima* trees are 6–25 m high with stem Ds up to 2.5 m (Kasaian et al. 2011). *J. foetidissima* is a tree species with the most significant D at 110 cm and had an MH of 14 m on the research site.

However, Carus (2004) measured an MH of 18 m and an MD of 62 cm in the Sütçüler region, which has 923.6 mm precipitation. Proutsos et al. (2021) measured *J. foetidissima* in the category of "very high trees (4%)" with an MH of 14 m and a D of 100 cm in Mt. Diti, where there was an average annual precipitation of 790.2 mm.

Influence of Site Conditions on Height and Diameter

According to the results of correlation analysis applied to both datasets, the most vital relationship was found in diameter and height values for all species (Dataset 1: *J. foetidissima* = 0.70, *J. excelsa* = 0.72, *P. nigra* = 0.77, *Q. cerris* + *Q. pubescens* = 0.53. Dataset 2: *J. foetidissima* = 0.73, *J. Excelsa* = 0.71, *P. nigra* = 0.70, *Q. cerris* + *Q. Pubescens* = 0.52). Oak showed low correlation coefficient value in both datasets. Height and diameter variables of *Q. cerris* + *Q. pubescens* were composed of young individuals. This result was expected because various scientific studies has proven a positive correlation between diameter and height (Sumida 2015). In comparison to others, *P. nigra* had the highest correlation coefficient related to MD–MH and M–D relationships. Even though the site conditions (human impact, woody plant coverage, tree density, altitude, exposure) showed logical correlations among themselves, the relationship between MH – MD and H–D differed based on species (Figure 4).

According to the results of variance analysis, *P. nigra* and *Q. cerris* + *Q. pubescens* comprised a group concerning human impact. Human impact and tree density created a minor correlation with the diameter and height values of *J. excelsa*, *P. nigra*, *Q. cerris* + *Q. pubescens*. While human impact values of *J. foetidissima* were correlated with MH – MD positively with a relatively high correlation coefficient (0.58 and 0.54), tree density values for MH – MD correlated negatively with a lower correlation coefficient (-0.43 and -0.41) (Figure 4). Human impact and tree density were directly related to human activity, such as habitat destruction, deforestation, fragmentation, and over-exploitation, which significantly affected forest structure and composition (Hauck and Lkhakvadorj 2013). Local people have used juniper branches as fodder for thousands of years all over Central Anatolia. Many studies have undoubtedly

revealed that human influence causes the reduction of tree density in the stands and destroys the tree occurrence over time (Ritchie and Roser 2021, Kahveci 2022). Meanwhile, it is remarkable that the correlation values of *P. nigra* related to human impact and tree density were very low. *P. nigra* had the highest tree density of all species (Figure 3).

The results of variance analysis presented that *P. nigra* and *J. excelsa* comprised a group concerning woody plant coverage, whereas *Q. cerris* + *Q. pubescens* and *J. foetidissima* comprised different groups. The results of correlation analysis showed that woody plant coverage was correlated with human impact negatively and with tree density positively with a relatively high correlation coefficient, which is normally expected. However, woody plant coverage of all species provided a very low correlation with MD and MH except for *J. foetidissima* (MD = -0.44 and MH = -0.39). It means, as woody plant coverage increases, MD and MD decrease, which is ordinarily difficult to explain. However, several studies have pointed out the effect of competition on tree radial growth (Buechling et al. 2017). The increase in competition increased tree height growth compared to no competition, and additional competition negatively impacted tree height (Aleinikovas et al. 2014).

The correlation analysis was applied to both datasets, and the most substantial negative relationship was found between height and diameter values of *J. excelsa* and altitude, although it had a low correlation coefficient value (MH = -0.47, MD = -0.42 in dataset 1 and H = -0.34 and D = -0.36 in dataset 2). However, height and diameter values of all other species in both datasets were negatively correlated with altitude, including very low correlation coefficient. Altitude is one of the critical physiographic factors affecting tree growth. The high altitudes generally show different environments, such as low air temperature and atmospheric pressure, precipitation prediction complexity, and extreme climates (Naud et al. 2019). In the Central Anatolia region, trees especially prefer to grow at a certain height because the humidity rate is very low in flat areas. Nevertheless, the climatic conditions at high altitudes are not favourable (Kahveci 2022). Özkan and Gülsoy (2008) observed a negative correlation between altitude and the height growth of *P. nigra* in Sütçüler. They concluded that the most critical

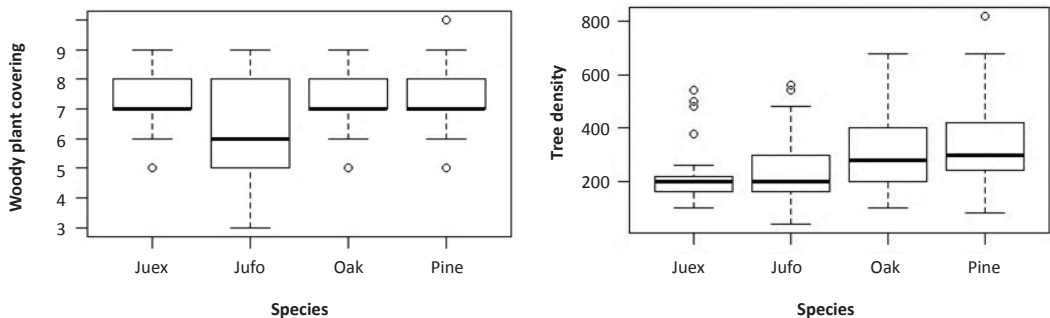


Figure 3. Visualised distribution of woody plant coverage and tree density values (TD = number of trees per plot*10,000/500) of four tree groups.

environmental factors in the height growth of *P. nigra* were altitude, slope, leaf-layer thickness, organic material in the soil, and humidity.

The correlation analysis applied to both datasets showed that *Q. cerris* + *Q. pubescens* provided a low correlation with site conditions, if any, with a low correlation coefficient value. A key question was whether these results were due to younger individuals of oak compared to the other tree species or because oak had a higher tolerance threshold to environmental conditions (Dreyer et al. 1992).

Colangelo et al. (2018) compared *Q. cerris* and *Q. pubescens* in terms of drought tolerance and complex responses to drought. They found different tolerances to the combined effects of climate and biotic stressors under the forecasted warmer and drier conditions. Abrams (1985) studied the relationship between age (D) and soil and slope as edaphic factors; the results showed that edaphic factors influenced the growth rates of oak species. Dai et al. (2020) also found a significant correlation ($p < 0.01$) between height and altitude for *Quercus mongolica*.

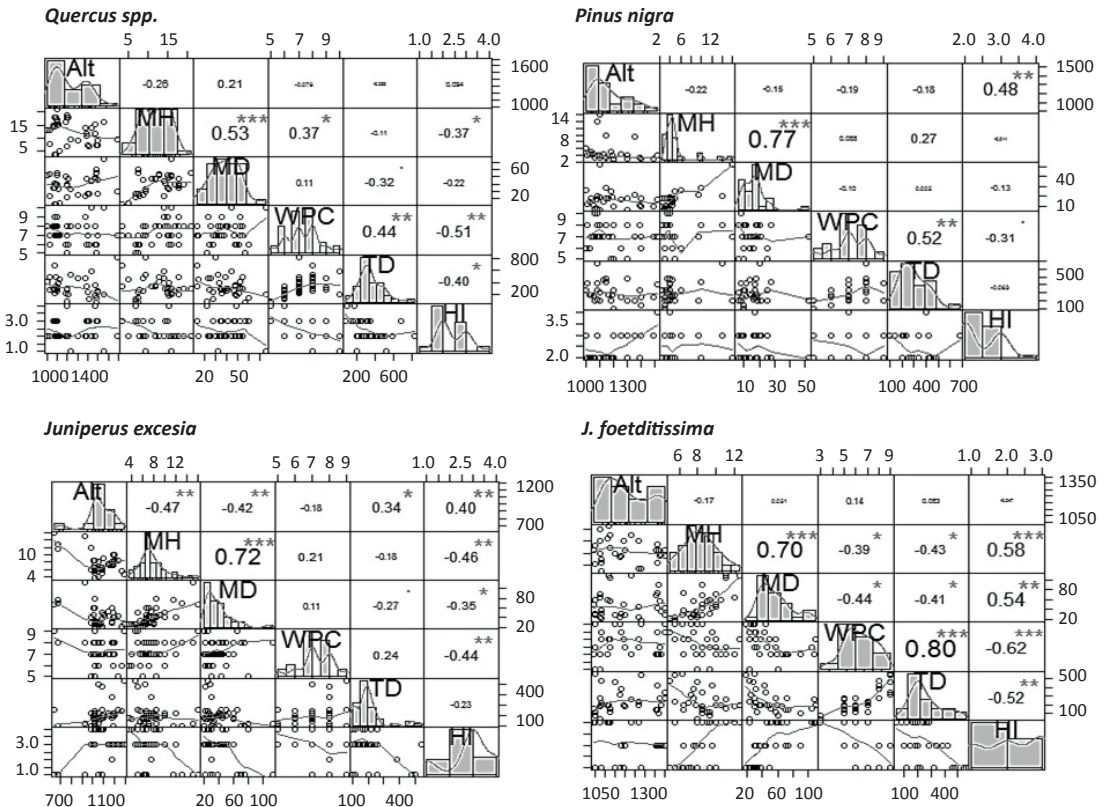


Figure 4. Comparison of the relationship between MH and MD and Altitude (Alt), woody plant coverage (WPC), tree density (TD), and human impact (HI) in species.

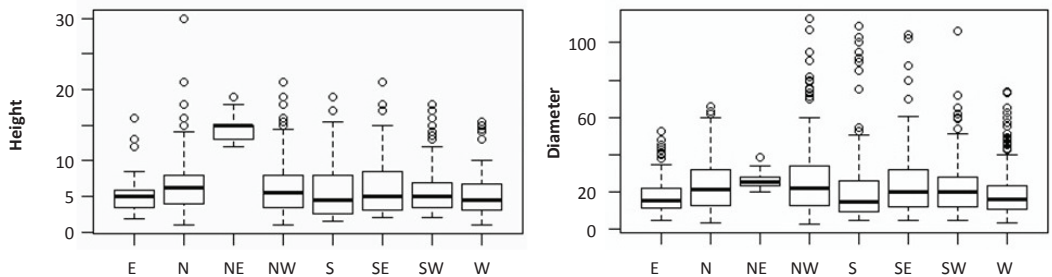


Figure 5. Visualised distribution of tree number related to H and D values in eight exposures (N - north; S - south; E - east; W - west; NE - northeast; SE - southeast; SW - southwest; and NW - northwest).

The effect of water stress usually increased on southern slopes because they received sunlight for a more extended period than other slopes and were thus more greatly affected by drought in Central Anatolia (Kahveci 2017). The juniper responded to changes in exposure and had narrower tree-ring width in southerly and westerly exposed slopes in Central Anatolia (Kahveci et al. 2018). Differences in the slope-related climate response of Scots pine were observed in Sweden (Kirchhefer et al. 2000). The results of variance analysis applied to the H and D values of the tree species and eight exposure supported these results. The total number of trees increased from NE (21), SE (132), E (148), W (172), SW (243) to N (250). While maximum heights are usually found on N-exposed slopes, maximum diameter was found on NW- and S-exposed slopes. When the average values were examined, it was observed that the highest MD and MH were found in the NE-exposed slopes (Figure 5). This led us to infer that on the southerly and westerly exposed slopes, drought worsened the conditions for growth, and the H growth remained at a certain level, whereas the D growth continued.

Height Prediction

The growth of trees, which occurs vertically and horizontally, is of great ecological significance. An adult tree is not only a reflection of the present conditions under which it grows, but it is also the result of all the genetic and environmental factors that have been operating as it has grown from seedling to maturity (Archibald and Bond 2003). Although H growth stops after a certain age depending on the tree's genetic code (Greenwood 1989), it is also affected by environmental conditions (Sumida et al. 2013). In this context, height growth may indicate various limitations in a tree's surrounding environment (Fulton 1998). Regression models applied to both datasets for height prediction were generated for tree species. The significant linear relationship ($p<0.001$) was between height and diameter in both datasets. Several equations relating to height prediction were compared, and the statistically most relevant equations were summarised in Table 3. The results of the regression equation applied to dataset 1 showed that the MH equations of each species involved different parameters. This led us to infer that the H of each tree was affected by different environmental factors while growing. In the equations created with dataset 2, the diameter was the only relevant variable for height prediction. Even in

this case, it was seen that the coefficients of the equations differed from each other.

CONCLUSIONS

Semi-arid regions have a significant effect on global atmospheric carbon variations. Central Anatolia is a semi-arid region of great significance for ecological research. The H-D growth of five native tree species from Central Anatolia and some of the affecting site conditions were investigated in this study. The most relevant relationship was found between height and diameter in all species. According to the results of this study, each tree species gave more or less different reactions to the site conditions (human impact, woody plant coverage, tree density, altitude, exposure). The MH and MD of *P. nigra*, *Q. cerris* + *Q. pubescens* did not have a significant relationship with site conditions, while those of *J. excelsa* were negatively related to the altitude and human impact. However, the MH and MD of *J. foetidissima* responded positively to human impact, and tree density and woody plant coverage negatively except for altitude. In this context, *J. foetidissima* might have the preferred specific locations for growing. The results of this study indicated that *P. nigra* had a shorter height in Central Anatolia than in areas with better environmental conditions. *J. foetidissima* and *J. excelsa* trees also had a shorter height, but the evidence was less clear. Height decline may be a reaction to drought, but the tree's genetic code plays an important role in tree behaviour in semi-arid regions. Exposure as an environmental factor affected both H and D growth. While the highest number of trees was found in N-exposed slopes, the largest number of MH and MD were found on NE-exposed slopes. The H-prediction equations showed that site conditions affected the height growth of natural species of Central Anatolia in different ways. However, none of them is as constant as the diameter. The results of this study might play a crucial role in maintaining ecological stability and preventing desert expansion and erosion. Although their distribution was relatively scattered, these four tree species are one of the most critical components of the local, fragile, terrestrial ecosystem. Classical forest management should change the current practice and focus on improving growth and reproduction, reducing competition, and reducing human impact on vulnerable forest resources.

Table 3. Height prediction equations for the four tree species in each dataset according to linear regression.

Tree	Dataset 1 (MD/MH+HI+WPC+TD+Alt)	Dataset 2 (D/H+Exp+Alt)
Oak	MH = -1.523126+0.2733588 MD+0.006511 TD	H = 1.0117+0.2262 D
Pine	MH = -8.594521+0.190724 MD+1.066217 WPC-0.009208 Alt	H = 2.08768+0.22887 D
Juex	MH = -5.62974+0.09203 MD-0.65648 HI	H = 2.830257+0.118686 D
Jufo	MH = -8.542158+0.041330 MD+0.584186 HI-0.002821 Alt	H = 4.645919+0.066251 D

Pini = *Pinus nigra*, Jufo = *J. foetidissima* Juex = *J. excelsa*, Quer = *Q. cerris* + *Q. pubescens*. Altitude (Alt), woody plant coverage (WPC), tree density (TD), and human impact (HI).

Author Contributions

GK conceived and designed the research, carried out the field and laboratory measurements, processed the data, drafted the manuscript, and wrote the manuscript.

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Conflicts of Interest

The author declare no conflict of interest.

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Collection of Experiences: 25 Years' Work on Seed Propagation of Allochthonous Woody Plants in Skopje and Their Possible Role in the Urban Landscape

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ABSTRACT

In the past decades, numerous landscaping efforts in the urban and suburban areas of Skopje have provided a diversified presence of allochthonous woody species. From the registered 206 allochthonous woody plants, in the past 25 years, 65 species (19 *Gymnospermae* and 46 *Angiospermae*) have been the focus of various research and monitoring efforts, including seed propagation and analysis of the generative propagation potential. Considering the variability in the experimental approach, we have performed an extensive literature revision and combined the results from the two in a summary of species that could be of potential interest in the green infrastructure in Skopje, due to their benefits (ornamental use and air pollution remediation) or risks (invasive and allergenic potential). We have also underlined the potential of using urban species as seed banks and species conservation, along with various services they could provide. To the best of our knowledge, this is the first attempt that summarizes the allochthonous woody species in Skopje along with the experience regarding their generative potential and possible use in urban forestry. As such, it serves as a base for future experimental research that could provide more information about the seed quality and the species' benefits in Skopje and the surrounding areas.

Keywords: generative propagation; urban forestry; dendroflora; air pollution remediation; ornamental use; invasive species; allergenicity

INTRODUCTION

The location and history of what is today the territory of North Macedonia (MKD) has provided suitable conditions for human settlements over two millennia. The high population rates and increased mobility in the region have also contributed towards frequent plants movement, primarily of edible and economically viable plants, e.g., *Ficus carica* L., *Morus*, *Cydonia oblonga* Mill. etc., as well as ornamental species. Some of these allochthonous species have adapted very well to the new ecological conditions, to such an extent that today they are considered as 'native' (Rizovska Atanasovska 1999). During the Ottoman Imperia rule over MKD (14th – 19th century), and especially towards the end of the 19th century, ornamental species were even more intensively introduced, especially in Skopje, the city that is still the capital today. At

the beginning of the 20th century, green areas in Skopje, today distinguished as urban green infrastructure, were established mainly as avenues and lawns, and later as city parks. For example, the location of the 'Skopje City Park' has not changed but its area has expanded over the last century. The subsequent expansion of Skopje, especially after the Second World War and the catastrophic earthquake in 1963, provided opportunities for more planned activities regarding the green infrastructure. However, this was followed by a period of more random and localized urban greenings, as smaller parks and similar types of green infrastructure, which accompanied the new residential and commercial zones (Rizovska Atanasovska and Vulgarakis 2014).

From a scientific standpoint, several planting activities have been noted as of particular interest, in the context of enriching the species diversity and planned introduction of

allochthonous species. The Botanical Garden, established by and in close proximity of the Faculty of Natural Sciences and Mathematics in the 1950s, provides a collection of curated and selected species cultivated in an open area. With support of the former Faculty of Agriculture and Forestry in the 1950s (today Faculty of Agricultural Sciences and Food, and the Hans Em Faculty of Forest Sciences, Landscape Architecture and Environmental Engineering (HEF), respectively), two live collections of autochthonous and allochthonous woody plants were established, (i) the Dendropark which surrounds the Faculties, and (ii) the arboretum in the Trubarevo village, located on the periphery of Skopje (Em et al. 1968).

Despite the large species diversity, few research efforts have focused on the allochthonous woody plants used in the green infrastructures. In the mid-1990s, the first comprehensive registration of allochthonous species in the urban and suburban areas in Skopje was established, listing 206 species (38 *Gymnospermae*, 168 *Angiospermae*) (Rizovska Atanasovska 1999). An ongoing collaborative project between Skopje and the United Nations aims to create a 'green cadastre' to analyse and monitor the greenery surface area of the city, but currently in Skopje there is little output, while in one of the neighbouring cities, similar attempts using modern geomatic techniques have been done (Mihajlovski et al. 2018). The reproductive potential of the present allochthonous woody plants has never been the primary research focus.

However, during the past 25 years, the period of establishment of some green areas which contain particular diversity, most trees and shrubs from the various green areas in Skopje (e.g., the Dendropark, the arboretum, the Botanical Garden, the City Park, etc.) have gone through their fructification stage. During this period, at the Department of Seed Science and Forest Stands at HEF, the reproductive material of some allochthonous woody plants has been periodically analysed. The aim of this paper is to summarise these experiences and provided insight into the species' characteristics, focusing on those of potential benefits or risks when used in the urban and suburban areas of Skopje. As urban trees can have a significant role as a seed source for both forest species in decline and non-native invasive species (Woodall et al. 2010), the output of this study is meant to serve as a tool for future selection of allochthonous woody plants and planning of relevant research activities for the local nurseries and urban greenery.

MATERIALS AND METHODS

The Skopje Region: Characteristics and the Seed Source Locations

The capital of North Macedonia, Skopje ([42°0'N 21°26'E](#)), is located in the Skopje Valley, at an elevation of 245 m asl, bordered by mountains on all four sides (west-Shar Mountains, south-Jakupica range, east-Osogovo range, north-Skopjska Crna Gora). The urban area of the city is mostly flat, spread on the north side of the Mount Vodno (1,066 m), the highest point inside the city borders. According to the last green census from 2012, two categories of urban greenery are present in Skopje, urban greenery with an area of 388 ha and peri-urban greenery with an area of 141 ha, in total covering 6% of the city area (EMA 2015). The complex geographical

attributes contribute to a particular microclimate of Skopje, a mix of continental and Mediterranean climate, exhibited by very hot and dry summers with short periods of heat waves, average cold and wet winters, with uneven spatial and temporal precipitation distribution (Donevska and Panov 2019). Late spring and early autumn frosts are an additional particularity that has been a major contributor towards the success/failure of plant establishment as part of the urban greenery (Shotaroska et al. 2019). Especially in the past decade, similarly to other highly urbanised cities, the air pollution during winter has had a severe negative impact on the climate and air quality (Martinez et al. 2018, Mirakovski et al. 2020).

Over the past 25 years (1997-2021), sampling was done at different locations in the city (Figure 1). The sampling sites have provided material for different allochthonous woody plants and include: the Dendropark of HEF located around the Faculty; the Arboretum of HEF in Trubarevo village; the Botanical Garden of the Faculty of Natural Sciences and Mathematics; several city parks (Skopje City Park, Park Zhelezara, Park Aerodrom); green areas around Lake Treska; park-forest Vodno and park-forest Gazi Baba; and, when available, residential gardens, and greenery of private and public enterprises.

Seed Handling and Analysis

Although the reproductive material was gathered over 25 years, the principles of the protocol were preserved, with appropriate modification depending on the species. The trees and shrubs that served as seed sources were subjected to regular phenological observations, across all seasons, throughout the years. Once the seeds, fruits, and/or cones had matured and ripened, they were appropriately collected. After an initial cleaning and drying, the collected material was processed, according to the species features and demands for sowing. The cones were dried outdoors or in a heated room until they opened. Based on the dormancy characteristics of the sowing material, a single treatment, or a combination of following pre-sowing treatments was performed:

- Pre-soaking in cold water (duration one-to-several days)
- Hydro-thermal procedure (immersing in boiling water for 10-40 seconds, then leaving in lukewarm water for 24 hours, followed by sowing)
- Pre-soaking in warm water (50-60°C) for several hours
- Maceration in 18-20% hydrogen chloride (HCl) for several hours
- Cold stratification in wet sand, on temperature of 3-8°C in the refrigerator or in outdoor objects (garage or soil trenches) for several weeks or months

The quantity of the collected material and seed/fruit features determined the type of subsequent analysis. When larger quantity was available, a laboratory-based germination rate or seed viability of the seeds was performed, by using a Jacobsen germination table or manual cutting, respectively. When smaller seed quantity, field germination rate was estimated by sowing the seeds in containers or in soil, at the nurseries of HEF (located in Skopje, Trubarevo and Krushevo) and in some nurseries of the PE National forests. As the ecological conditions of these

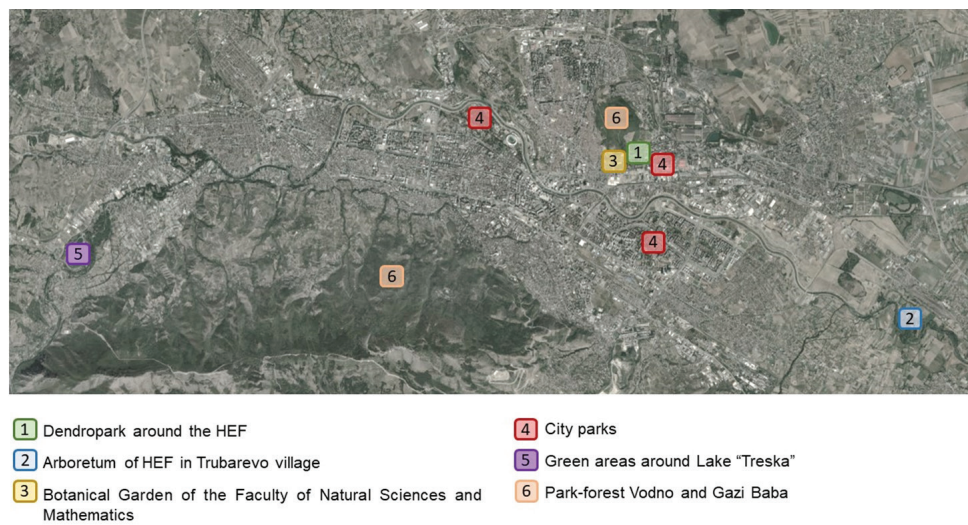


Figure 1. Locations in Skopje and the surrounding area where the samplings took place.

nurseries significantly differ (location altitude between 240 – 1,300 m), the preferred ecological conditions of the species were taken into consideration. For the majority of the seed material, the sowing was done in the spring season, except for several species that require sowing in the autumn season (*Quercus*, *Juglans*, *Ginkgo biloba* L.). For all species, one-year old (1+0) seedlings were measured for shoot height (SH) and root collar diameter (RCD). Based on the list of analysed species, a literature review was done in order to separate them in one of the predetermined categories. In the context of Skopje, four categories were defined, two regarded as negative (potential species of risk) and two regarded as positive (potential species of benefit) (Figure 2).

RESULTS

In the present study, we report the propagation characteristics of seeds from allochthonous woody plants, collected in the Skopje region as part of different sampling efforts between 1997 and 2021. These sampling efforts resulted with 65 species, 19 *Gymnospermae* and 46

Angiospermae, that were further categorized based on their level of presence. The germination or viability was also accounted (Table 1). The data shows that of the listed *Gymnospermae* species, all are coniferous except *Ginkgo biloba*, while all of the *Angiospermae* species are broadleaf species. The sampling efforts have been more inclusive towards broadleaf trees and shrubs, as they generally prevail in the green areas in Skopje (Rizovska Atanasovska 1999) and partly because the cone collection for the case of conifers as high trees is extremely difficult. Low germination/viability rate of the seeds was registered in coniferous species which are characterized by such feature (e.g., *Cupressus* (Tadros et al. 2010), *Pseudotsuga*), but also in solitary broadleaf trees, where probably an inbreeding, i.e. a self-pollination occurs (e.g., *Sterculia platanifolia* L. (Brizicky 1966), *Pterocarya fraxinifolia* Poir., *Idesia polycarpa* Maxim., *Acer davidii* Franch.). In *Acer davidii* almost every year abortion was registered, i.e., the rejection of immature fruits, shortly after the insemination in May (Kolevska, personal communication). The selection of suitable pre-sowing treatment is a crucial step for insuring higher germination rates. For some species that are generally more studied, the

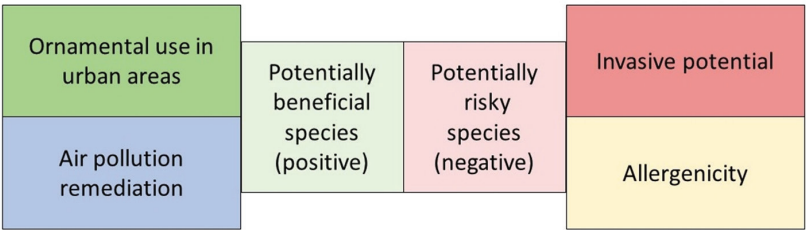


Figure 2. The four categories considered for grouping the species.

applied adequate pre-sowing treatments have contributed to higher laboratory and field germination rates (e.g., *Acer negundo* L., *Fraxinus americana* L., *Gleditsia triacanthos* L., *Robinia pseudoacacia* L.) (Supplementary Material 1). The limited knowledge available and experience regarding some

of the studied allochthonous species might also contribute to reduced germination rates, especially during the selection of the pre-sowing treatment. The literature revision allowed us to assign at least one category to 39 species (Table 1) (Figure 3).

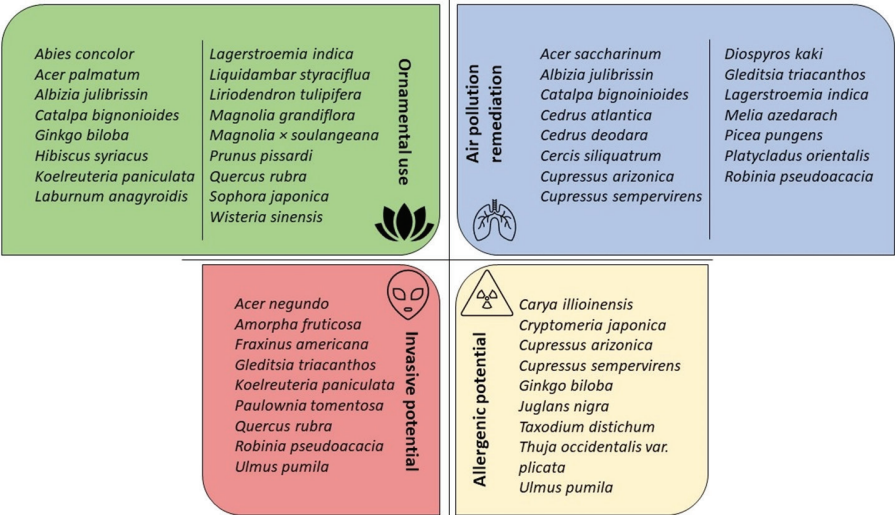


Figure 3. Woody plant species of interest for Skopje from the four categories of species of interest, ornamental use, and air pollution remediation as those of beneficial interest, and invasive potential and allergenic potential as those of risky interest. The included species are the overlaps between the revised literature and the studied species.

Table 1. Characteristics of sampled allochthonous woody plants in Skopje (1997-2021).

Species	Level of presence ¹	Germination or viability ²	Risk/benefit categories			
			Invasive potential	Allergenicity	Ornamental use	Air pollution remediation
<i>Cryptomeria japonica</i> D.	RARE	VL		x		
<i>Calocedrus decurrens</i> Torr.		L				
<i>Pinus wallichiana</i> Jacks.		M				
<i>Taxodium distichum</i> L.		M		x		
<i>Thuja occidentalis</i> var. <i>plicata</i> Donn.		M		x		
<i>Abies concolor</i> Lindl.	COMMON	L			x	
<i>Chamaecyparis lawsoniana</i> Murray		L				
<i>Larix decidua</i> Mill.		L				
<i>Pseudotsuga menziesii</i> Mirb.		L				
<i>Cedrus deodara</i> Roxb.		M				x
<i>Cedrus atlantica</i> Endl.		M				x
<i>Ginkgo biloba</i> L.		H		x	x	
<i>Picea pungens</i> Engelm.		H				x
<i>Picea omorika</i> Pančić	ABUNDANT	H				
<i>Pinus strobus</i> Thunb.		H				
<i>Cupressus arizonica</i> Greene		L		x		x
<i>Cupressus sempervirens</i> L.		L		x		x
<i>Thuja occidentalis</i> L.		L				
<i>Platycladus orientalis</i> L.		M				x

Table 1. (continue) - Characteristics of sampled allochthonous woody plants in Skopje (1997-2021).

	Species	Level of presence ¹	Germination or viability ²	Risk/benefit categories			
				Invasive potential	Allergenicity	Ornamental use	Air pollution remediation
	<i>Acer buergerianum</i> Miq.		VL				
	<i>Sterculia platanifolia</i> L.		VL				
	<i>Acer davidii</i> Franch.		L				
	<i>Exochorda racemosa</i> L.		L				
	<i>Idesia polycarpa</i> Maxim.		L				
	<i>Melia azedarach</i> L.		L				x
	<i>Pterocarya fraxinifolia</i> Poir.		L				
	<i>Alnus cordata</i> Lois.		M				
	<i>Aronia melanocarpa</i> Michx.	RARE	M				
	<i>Chimonanthus praecox</i> L.		M				
	<i>Citrus trifoliata</i> L.		M				
	<i>Maclura tricuspidata</i> Carrière		M				
	<i>Parrotia persica</i> DC.		M				
	<i>Aesculus glabra</i> Willd.		H				
	<i>Carya illinoensis</i> Wangenh.		H		x		
	<i>Juglans mandshurica</i> Maxim.		H				
	<i>Quercus aegilops</i> L.		H				
Angiospermae	<i>Acer palmatum</i> Thunb.		L			x	
	<i>Diospyros kaki</i> L.		L				x
	<i>Lagerstroemia indica</i> L.		L			x	x
	<i>Liquidambar styraciflua</i> L.		L			x	
	<i>Liriodendron tulipifera</i> L.		L			x	
	<i>Albizia julibrissin</i> Durazz.		M			x	x
	<i>Amorpha fruticosa</i> L.		M	x			
	<i>Campsis radicans</i> L.		M				
	<i>Koeleruteria paniculata</i> Laxm.		M	x		x	
	<i>Laburnum anagyroides</i> Medik.		M			x	
	<i>Maclura pomifera</i> Raf.		M				
	<i>Magnolia grandiflora</i> L.		M			x	
	<i>Magnolia × soulangeana</i> hort.		M			x	
	<i>Wisteria sinensis</i> Sims.		M			x	
	<i>Acer saccharinum</i> L.		H				x
	<i>Catalpa bignonioides</i> Walter		H			x	x
	<i>Cercis siliquastrum</i> L.		H				x
	<i>Juglans nigra</i> L.		H		x		
	<i>Paulownia tomentosa</i> Thunb.		H	x			
	<i>Pistacia vera</i> L.		H				
	<i>Quercus rubra</i> L.		H	x		x	
	<i>Ulmus pumila</i> L.		H	x	x		
	<i>Prunus pissardi</i> Carrière		M			x	
	<i>Sophora japonica</i> L.		M			x	
	<i>Acer negundo</i> L.		H	x			
	<i>Fraxinus americana</i> L.	ABUNDANT	H	x			
	<i>Gleditsia triacanthos</i> L.		H	x			x
	<i>Hibiscus syriacus</i> L.		H			x	
	<i>Robinia pseudoacacia</i> L.		H	x			x

¹ Rare - solitaires or only several individuals; Common - present in many green areas; Abundant - very often present in green areas. ²VL - very low (<0%); L - low (10-40%); M - medium (40-60%); H - high (> 60%).

DISCUSSION

In urban and suburban areas, vegetation provides a myriad of services, i.e., microclimate regulation, moisture increase, air cooling, oxygen supply, noise reduction, improvement of the radiation regime, suspended particulate matter filtration, wind reduction or redirection, supporting the diversity of arthropod and vertebrate communities, etc. (Attorre et al. 2000, Schmitt-Harsh et al. 2013, Irmak et al. 2018, Frank et al. 2019, Rita Baraldi et al. 2019, Csontos et al. 2020, Ribeiro et al. 2020, Masalova et al. 2021). Furthermore, trees also have a mnemonic and social role, and green infrastructure has the potential to give new life to the city after a catastrophic event, build solidarity and commemorate the past (Magnus and Sander 2019). All these aspects underline the sustainable and multifunctional role of the urban vegetation. During the historical and recent development of Skopje, a variety of woody plant have been introduced. Thus, a particular urban landscape has been established, which has not been thoroughly examined, and often not thoroughly planned. Urban areas that are not characterized by a great species diversity can undergo a great reduction of the green areas, due to unforeseen negative conditions and upcoming climatic challenges (Sjöman et al. 2010). Therefore, species selection needs to be based on their ability to provide various ecosystems services, as well as their tolerance to climatic changes, as diversified urban forests hold higher potential (Sjöman et al. 2021). However, the relationship between urban areas and the urban vegetation is dynamic and reciprocal. Appropriately selected species can satisfy the main requirements of the urban areas, by demonstrating capacity for resistance of the anthropogenic pressures and meeting the aesthetic needs (Masalova et al. 2021). Furthermore, urban areas can serve as easily accessible and managed points of *in situ* conservation of potentially endangered species (Pan et al. 2019) or species of particular interest whose genetic material can be used in local nurseries for the production of better adapted individuals to the particular eco-climatic conditions (Sjöman and Watkins 2020). The lack of knowledge regarding the species performance in urban areas has a negative impact, not only on the potential for suitable further selection of species, but also as a significant financial burden on the public budget, which can be remediated with appropriate species-site selection (Percival et al. 2006). It is therefore of vital importance that future selection is made by experts and that the already established green infrastructure is monitored, appropriately managed, and maximally used.

In the present study, we have provided an overview of 25 years of experience with generative propagation of allochthonous woody plants present in Skopje, as a first attempt to summarize and contribute towards the body of knowledge. In particular, considering the limitations of the study, we have focused on 65 species in terms of their presence and seed germination/viability, as well as their potential benefits and/or risks in Skopje, as part of the green infrastructure. Although some of the examined allochthonous species can be more successfully propagated by vegetative methods, we have focused solely on the potential for generative propagation from various seed sources from green areas of Skopje and its surroundings (Supplementary Material 1).

The Importance of Quality Planting Material

Well-planned, executed and maintained urban greenery can yield numerous ecological, economic and social benefits, thus increasing the life quality in cities and urbanized areas (Allen et al. 2017). However, the highest percentage of plant mortality, up to 90%, takes place during the earliest life stages (Labatore et al. 2017). This is especially the case for urban trees in the first two-three years after transplantation and it can be greatly reduced by accounting for the planting season, site selection and management (Koeser et al. 2014). Prior to that, the nursery stage, i.e., the production steps of trees and shrubs used for urban greenery impacts numerous seedling properties, e.g., root architecture, fine root hydraulic conductance, stem girdling root production, plant establishment and growth response, nutrient uptake and post-transplant irrigation requirements, pathogen susceptibility, which significantly impact the success rate of the post-transplant establishment of urban trees (Allen et al. 2017). Furthermore, it is also important to account for continuous tree recruitment, i.e., gathering, analysing and seed selection, in order to ensure a sustainable urban vegetation structure (Labatore et al. 2017).

In MKD, through decades, the ecological as well as the political and economic changes and challenges (Arsovski et al. 2018) have concurrently impacted the nursery production of seedlings of allochthonous trees and shrubs, used for reforestation and ornamental purposes. Between 1995 and 2000, the average annual production of seedlings in forest nurseries was ca. 6 million, of which 78% coniferous and 22% broadleaf species (Kolevska 2005). Among the coniferous, 4 species were autochthonous and 6 allochthonous. From the allochthonous species, *Cupressus arizonica* Greene (ca. 600,000 seedlings) and *Pseudotsuga taxifolia* Lamb. (390,000 seedlings) were more dominant, and sporadically, depending on seed supplies, seedlings of *Cedrus deodara* Roxb., *Chamaecyparis lawsoniana* Murray, *Platycladus orientalis* L. and *Sequoiadendron giganteum* Lindl. were produced in significantly smaller quantities (ca. 1,000 – 14,000 seedlings). Among the broadleaf species, 5 species were autochthonous and 3 allochthonous, with an absolute domination of *Robinia pseudoacacia* (average production of 1 mil. seedlings), while *Fraxinus americana* and *Acer negundo* were produced in much smaller quantities (50,000 and 18,000 seedlings, respectively). In the following decade, a general decrease of the number of produced seedlings and afforestation rates had taken place, with a preference towards allochthonous species. Kolevska et al. (2017) note that in 2016, from a total of 22 nursery-produced species, 11 were allochthonous. Out of those, 63% were coniferous (*Cupressus arizonica* – 276,000 seedlings, *Pseudotsuga taxifolia* – 234,000 seedlings, *Platycladus orientalis* – 1,500 seedlings) and 37% broadleaf (*Robinia pseudoacacia* – 900,000, *Fraxinus americana* – 327,000 seedlings, and other allochthonous species as *Acer negundo*, *Juglans nigra* L., *Albizia julibrissin* Durazz., *Cercis siliquastrum* L., *Koeleruteria paniculata* Laxm. and *Sophora japonica* L. in quantities of 1,300-8,000 seedlings), and the majority of these species were produced primarily for ornamental use (Kolevska et al. 2017). The ornamental plant production in nurseries in MKD is particular for other reasons as well. During the 1980s and the 1990s, most ornamental tree species were produced from seed, with a strong preference towards autochthonous

species and only certain allochthonous species (e.g., *Abies concolor* Lindl., *Cupressus arizonica*, *Picea pungens* Engelm., *Cedrus deodara*, *Cedrus atlantica* Endl., *Pinus ponderosa* Douglas ex Loudon, *Pinus strobus* Thunb., *Pinus wallichiana* Jacks.) (Kolevska, 1995 – unpublished data). However, due to globalization, the past 20 years have been marked with a rapid production decline of ornamental seedlings in the country and import of mainly big transplants, which has severely affected the domestic ornamental nurseries with long and successful tradition in generative propagation and production of big seedlings (Kolevska, personal communication). This situation marks yet another aspect that needs to be taken into consideration, the inclusion of local nurseries in the process of green infrastructure establishment and the knowledge availability regarding allochthonous seed preference for growing conditions and management.

The nursery-to-landscape transplantation is extremely stressful and critical for the plant establishment and growth resumption (Franco et al. 2006). The container size and type are another delimitating factor in the plant production process and the trend of reducing the size of the container, i.e., reducing the production costs per plant, affects the growth and the development of plants, especially the root characteristics in the case of woody plants (Franco et al. 2006). Indeed, we observed differences in the quality features in 1+0 seedlings, i.e., shoot height and root collar diameter (Supplementary Material 1), which can vary significantly from nursery to nursery as a factor of both ecological characteristics of the nursery and the management practices. This is especially notable when comparing seedlings from the same species grown as bare roots in different nurseries. For example, in *Albizia julibrissin* seedlings grown as bare roots in similar ecological conditions, SH varies between 15 cm and 180 cm; in *Pseudotsuga taxifolia* SH varies between 7 cm and 35 cm; in *Robinia pseudoacacia* SH varies between 23 and 240 cm (Supplementary Material 1). When the seedlings were grown in containers, bigger and thicker seedlings were developed and less differences were observed between plants grown in different nurseries. Considering that living plant collections from botanical gardens and green urban areas can provide experience on the development of allochthonous species as well as reproductive material (seeds) (Sjöman and Watkins 2020), the study was aimed towards woody plants that could be produced in MKD nurseries suitable for further use in urban forestry, especially due to their properties for climate mitigation and the increase of the air quality.

Different Outcome from the Use of Allochthonous Woody Species

The allochthonous trees and shrubs that have been examined in the past 25 years (Table 1, Supplementary Material 1) provided a list of species that we are interested to explore further, due to their benefits and/or risks for the urban greenery in Skopje. As there is very limited research regarding the experience in Skopje, we employed a literature revision analysis to gather external experience and potential references for future selection and management of the species. As described in the previous sections, we predefined two categories, the potentially risky species

(negative impact) and the potentially beneficial species (positive impact).

Potentially Risky Species – Negative Impact

Within the group of species with potential negative impact in the context of urban areas, we defined two groups, species with invasive potential and species with allergenic potential, both of relevance in the context of Skopje. The invasive potential of allochthonous species is an active discussion in forestry (Pötzelberger et al. 2020b, Bindewald et al. 2021, Dimitrova et al. 2022), but it is also important in urban areas. Over the centuries and with the increase of anthropogenic mobility, due to their exotic nature, many allochthonous species were primarily introduced in urban areas from where they could disperse and employ both active and passive invasive mechanisms (Trusty et al. 2007, Deparis et al. 2022). While active invasion is a result of the introduction of species that spread quickly and easily under suitable new conditions, passive invasive mechanisms refer to species that have been present for a long time and/or are present in considerable numbers, and thus become more dominant (Trusty et al. 2007). Regardless of the mechanism, the risks of these allochthonous species is a potential escape from the borders of their cultivation in (semi) natural areas and the establishment of self-sustaining stands where they might exhibit invasive behaviour (Csontos et al. 2020). Which species exhibit invasive behaviour depends not only on their biological characteristics, but also on the environmental conditions. For several of the studied species, the invasive behaviour and invasive potential have been noted, both in MKD and across Europe (e.g., *Amorpha fruticosa* L., *Koeleruteria paniculata*, *Maclura aurantiaca* Nutt., *Quercus rubra* L., *Fraxinus americana* and *Gleditsia triacanthos* (Kolevska and Acevski 2005, Krumm and Vitková 2016, Dimitrova et al. 2022)). Considering that these species have been characterized either as 'common' or 'abundant', and with medium or high germination rate (Table 1), they impose a need for close monitoring in urban green areas. As the literature notes, *Gleditsia triacanthos* and *Koeleruteria paniculata* have been characterized as being able to establish high-density soil seed banks underneath the canopy with viable, hard-coated seeds which can be further dispersed by birds and mammals to great distances (Csontos et al. 2020, Ljubojević et al. 2021). *Paulownia tomentosa* Thunb. has already been recognized as a colonizer, mainly of disturbed urban areas in Central Europe, but its future expansion due to the climatic changes is probable (Essl 2007). *Amorpha fruticosa* is a registered invasive alien species in Europe, with high tolerance to various habitat conditions and strong colonization potential, especially in riparian areas (Kozuharova et al. 2017, Boscutti et al. 2020). In Skopje, invasive species that successfully colonize riparian areas are a significant threat to the riverbanks of the Vardar River. While in the more urbanized zones (the central and adjacent regions) riverbanks are regularly managed, in the peripheral city areas and the left bank, there are considerably less organized activities to maintain and select the flora. From the species noted as present in Skopje, potentially risky is *Acer negundo*, with demonstrated preference for these types of habitats in Central and South Europe and a threat for the *Salix* and *Fraxinus* species (Saccone et al. 2010,

Erfmeier et al. 2011, Porté et al. 2011, Sikorska et al. 2019, Deparis et al. 2022). Furthermore, rivers can easily transport vegetative parts and seeds of invasive species and contribute to active invasion of different ecosystems, something which has indeed been detected for *Amorpha fruticosa* and *Acer negundo* in the north, in neighbouring Serbia (Radovanović et al. 2017). The near-natural habitat colonization has been seen in the case of the severely invasive *Ailanthus altissima* Mill. (Essl 2007) as well as *Ulmus pumila* L. (Lykholat et al. 2018). *Robinia pseudoacacia* is another species which has emerged as a serious threat in Europe (Vitková et al. 2020) and while some areas such as railways could be a suitable habitat where the black locust can provide ecological and aesthetic services, its interaction potential with other urban and ecological patterns needs to be further examined (Deparis et al. 2022). In the context of MKD, the contrasting information between the species produced in nurseries and the legal restrictions is also interesting. Pötzelsberger et al. (2020a) report that for species such as *Acer negundo* and *Robinia pseudoacacia* soft ban laws exist, yet the on-field situation in nurseries is different. This raises concerns, since both species exhibit high germination rates and viability (Table 1). The impact of invasive allochthonous species on recreational ecosystem services that are urbanized on various levels is an emerging field of interest as these species also affect the use of the recreational areas, thus influencing how people see them and how they value them (Sikorska et al. 2019). Prevention by regular and planned management, raising awareness and biological investigations of the seeds are crucial steps to reduce the potential risk of increased presence of invasive species in both urban and suburban environments (Csontos et al. 2020, Deparis et al. 2022). Alternative scenarios for using invasive species have also been considered, e.g., using various parts of *Amorpha fruticosa* as a resource for remedial purposes (Kozuharova et al. 2017) and *Koeleruteria paniculata* seeds as a novel feedstock for biodiesel production (Ljubojević et al. 2021).

The allergenic potential of species used in urban areas is relevant since increased pollen concentrations negatively impact the air quality and human health. The increase of allergies and pollen distribution has also been noted in Skopje, and it is expected to rise along with the predicted temperature increase (Kendrovski et al. 2014). Trying to fulfil aesthetic and recreation needs along with rushed species selection has led to use of both autochthonous and allochthonous woody plants with high allergenic potential in the urban areas (Velasco-Jiménez et al. 2020). Velasco-Jiménez et al. (2020) list paper mulberry, Australian pine, cypress, olive, plane tree, poplar and elm as most allergenic species, but Cariñanos and Marinangeli (2021) have comprised an extensive overview in this regard for 150 species in the Mediterranean region. Considering the climatic similarities with Skopje and the surroundings, we extracted the overlap between their list and our sampled species, concluding that attention in this aspect needs to be brought to the following species: *Cryptomeria japonica* D., *Cupressus arizonica*, *Cupressus sempervirens* L., *Ginkgo biloba*, *Taxodium distichum* L., *Thuja occidentalis* var. *plicata* Donn., *Carya illinoensis* Wangerh., *Juglans nigra*, and *Ulmus pumila*. While the majority of these species are

coniferous, and only three of them are broadleaf species (*Carya illinoensis*, *Juglans nigra*, and *Ulmus pumila*), there is large variability in terms of the germination rate and occurrence in the city (Table 1). Indicatively, species that showed to be more common and had higher germination rate are to be more closely monitored, e.g., *Ginkgo biloba*, *Juglans nigra*, and *Ulmus pumila* (Lorenzoni-Chiesura et al. 2000). *Cryptomeria japonica* is known to be a species with high allergenic potential in Japan (Nakamura and Xiu 2012), but we found no relevant literature regarding the other species. We can therefore recommend further research into the pollen production of the allochthonous species present in Skopje, and predictive models that use the trunk size, canopy area and height, as well as the number of flowers per tree, the number of anthers per flower and the number of pollen grains per anther (Katz et al. 2020).

Potentially Beneficial Species – Positive Impact

Within the group of species with potentially positive impact in the context of urban areas, we defined two groups, species of particular ornamental value for urban areas and species that can be used for air pollution remediation. All woody species, depending on their specific characteristics, have their own role in the landscape design of urban spaces. The ornamental value of many trees is often based on their special colour, shape and structures and many of them possess ornamental value because of their particular colour of leaves, flowers and/or other organs at certain developmental stages (Wang et al. 2014). In our study, we underline some species whose ornamental value is more emphasized due to their flowers (*Albizia julibrissin* Durazz., *Catalpa bignonioides* Walter, *Hibiscus syriacus* L., *Koeleruteria paniculata*, *Laburnum anagyroides* Medik., *Lagerstroemia indica* L., *Liriodendron tulipifera* L., *Magnolia grandiflora* L., *Magnolia × soulangeana* hort., *Sophora japonica* or *Wisteria sinensis* Sims.) and fruits (*Catalpa bignonioides*), their crown shape (*Abies concolor*, *Albizia julibrissin* and *Catalpa bignonioides*), and the shape and/or colour of leaves (*Acer palmatum* Thunb., *Albizia julibrissin*, *Catalpa bignonioides*, *Ginkgo biloba*, *Liquidambar styraciflua* L., *Liriodendron tulipifera*, *Prunus pissardii* Carrière and *Quercus rubra*). For example, autumn leaf colour makes sweet gum (*Liquidambar styraciflua*) a favourable ornamental tree species for landscape beautification and urban greening (Wang et al. 2014), red leaves in autumn increase ornamental value of *Quercus rubra* (Dyderski et al. 2020) and golden leaves in autumn make *Ginkgo biloba* more attractive. *Acer palmatum* is a small woody tree widely ornamentally used for leaf shapes and colour (Li et al. 2015). *Catalpa bignonioides* is a tree with an attractive ornamental value and compact shape (Pošta et al. 2021) and its decorativeness is also increased due to the unusual shape of the leaf. The use of *Prunus pissardii* is often considered in the context of contrast in landscape design, specifically, the contrast combinations of leafage colours in red-garnet (*Prunus pissardii*) with the silver colour of *Elaeagnus argentea* Pursh. (Anca 2012). Indeed, contrast is one of the most used criteria in landscape designing as contrasts of forms, and especially of colours, assure spectacular effects to the entire vegetal composition (Anca 2012). Another plant that is decorative not only for

its leaves but also for its flowers is *Albizia julibrissin*, a small deciduous tree growing up to 5–16 m (16–52 ft) tall which is widely planted as an ornamental plant in parks and gardens, grown for its leaf texture and flowers (Roy et al. 2016). The decorativeness of the leaves is not based only on the non-specific form (the leaves are bipinnate), but also on their ability to slowly close during the night and during periods of rain, and the leaflets bowing downward, hence its modern Persian name *shabkhosb* which means "night sleeper" (Roy et al. 2016). *Liriodendron tulipifera* is also an ornamental tree thanks to the extraordinary tulip-shaped flowers and goose web-like leaves (Wei et al. 2022). During previous urban greening activities in Skopje, these attributes have indeed been taken into consideration for the species selection (Figure 4). Furthermore, we can observe that when species of higher ornamental value have been selected, the management has been more focused and consistent, providing for higher greenery quality and longer lifespan.

One of the ecosystem services of woody plants is also air pollution remediation, by a dual strategy. Trees, both through their above- and below ground organs, are able to (1) significantly contribute to the reduction of air pollution by accumulation of metals and pollutants (Li et al. 2019, Simon et al. 2021) and (2) serve as long-term indicators of air pollution due to the direct and indirect uptake of atmospheric pollutants (Cui et al. 2022). Different woody species have different capacities, usually determined by their morphological characteristics (e.g., leaves with big surface

area such as *Catalpa bignonioides* or compound leaves such as *Albizia julibrissin*) (Vordoglou et al. 2019). Furthermore, species tolerant to air pollutants are those that can serve as sinks or traps for atmospheric particulate matter (PM), while species sensitive to air pollutants can serve as indicators (Ogunkunle et al. 2019, Simon et al. 2021). The rise of air pollution in cities has also raised interest in the suitable species selection for these purposes. However, it is also worth noting that one species cannot do everything, which further emphasises the need for using different species which are able to be more efficient in the accumulation of different pollutants. For example, in a case-study in Hungary, Simon et al. (2021) found *Robinia pseudoacacia*, *Tilia europaea* L., *Acer platanoides* L., *Fraxinus excelsior* L., *Betula pendula* Roth., and *Celtis occidentalis* L. to be a suitable sensitive indicator species of air pollution, while *Acer saccharinum* L., *Betula pendula* and *Platanus x acerifolia* Willd. were recommended as pollutant-accumulator species. Several other studies provide details regarding the accumulator species. Yang et al. (2015) provide a comprehensive list of the most frequently occurring species in urban areas and their PM removal efficiency as well as negative impact and suitability for urban environments, concluding that from the most frequent species, *Platanus x acerifolia*, *Acer saccharinum* and *Gleditsia triacanthos* exhibit above-average efficiency. In more detail, *Albizia julibrissin* has exhibited higher capacity for capturing manganese and zinc, while *Cupressus arizonica* captured copper, lead, cadmium, chromium and nickel (Samara and



Figure 4. Examples from the use of oriental tree species due to their particular characteristics in urban areas in Skopje. (a) and (b) *Ginkgo biloba* leaves, leaf colour in autumn as an ornamental point of interest; (c) *Prunus pissardii* in bloom, flowers as an ornamental point of interest; (d) *Magnolia x soulangeana* in bloom, flowers as an ornamental point of interest; (e) *Prunus pissardii*, leaf color throughout the year, as an ornamental point of interest. Photo credit: Viktorija Brndevska Stipanović, Skopje 2022.

Tsitsoni 2014). *Platycladus orientalis* L. could also aid in cadmium mitigation (Cui et al. 2022). Some species have also been determined as more resistant to air pollution and as such are recommended for heavily polluted (semi) urban areas, e.g. *Robinia pseudoacacia*, *Cupressus arizonica* and *Platanus orientalis* L. at industrial sites (Isinkaralar 2022b), *Magnolia denudata* Desr., *Diospyros kaki* L., *Ailanthus althissima*, *Fraxinus chinensis* Roxb. and *Rosa chinensis* Jacq. along heavy traffic roadsides (Zhang et al. 2016). The sensitivity of other species makes them more suitable to be used for biomonitoring and a combined list could include *Ailanthus altissima*, *Platanus orientalis*, *Cedrus deodara*, *Cupressus sempervirens*, *Pinus pinea* L., *Nerium oleander* L., *Ligustrum ovalifolium* Hassk., *Pittosporum tobira* Aiton., *Lagerstroemia indica*, *Melia azedarach*, *Acer pseudoplatanus*, and *Quercus ilex* L. (Pignata et al. 1999, Rucandio and Petit-domínguez 2011, Bozdoğan 2016, Liang et al. 2017, Shrestha et al. 2021, Emel and Hakan 2022). From the viewpoint of species-heavy metal pollutant preference, in the case of *Cedrus atlantica* gradual concentration increase of lead and cadmium has been noted due to the increase of the number of vehicles (Savas et al. 2021, Isinkaralar 2022a). The annual rings of *Cupressus arizonica* are suitable biomonitors for iron, but not for lithium and chromium (Cesur and Zeren 2022). *Sabina chinensis* Antoine. has also exhibited pollution sensitivity (Cui et al. 2022), along with *Betula pendula* which is suitable regarding cadmium, while *Aescullum hippocastanum* L. could be efficiently used for monitoring lead pollution (Gh et al. 2012). *Picea pungens* has been noted for chromium and zinc (Sulhan et al. 2022), while *Cercis siliquatum* is suitable for lead and cadmium but not for iron (Yaşar et al. 2010, Hatamian et al. 2019). Liang et al. (2017) also found *Cedrus deodara* to be suitable for lead and cadmium, while they determined *Nerium indicum* Mill. and *Platanus acerifolia* as most suitable for copper and *Pittosporum tobira* Aiton. for zinc and cadmium. In the context of urban greenery, an important consideration is how air pollution can negatively impact seed germination/viability (Tadros et al. 2010) as seen in *Pinus nigra* Aiton. and *Cupressus arizonica* (Babapour et al. 2020).

The overlaps between the studied woody plants and the literature indicate that regarding air pollution mitigation for Skopje, species of special interest could be *Cedrus deodara*, *Cercis siliquatum*, *Cupressus arizonica* and *C. sempervirens*, *Lagerstroemia indica*, *Melia azedarach*, *Picea pungens*, and *Robinia pseudoacacia* for biomonitoring different pollutants, and species such as *Acer saccharinum*, *Albizia julibrissin*, *Cupressus arizonica*, *Diospyros kaki*, *Gleditsia triacanthos*, and *Platycladus orientalis* as sinks for certain pollutants. The air pollution patterns in Skopje follow both a diurnal and seasonal cycles, with pollution peaks in the autumn and winter, in the morning and the late evening, and being a particularly big issue in the winter months, mainly due to the heating (Mirakovski et al. 2020). This needs to be considered for future species selection for air pollution mitigation, as mainly *Gymnospermae* species would be able to fulfil the function during the pollution's peaks.

Urban Seed Sources: Impacts of Climatic Changes, Urbanization, and Species Selection in Skopje

The climatic changes and increased levels of urbanisation have contributed to the early spring phenomenon in cities

in comparison with the surrounding countryside, which impacts the trees phenology and furthermore the arthropod communities, birds migrations and breeding, and the overall vegetation composition (Shustack et al. 2009, Stanciu et al. 2021). Over the 25 years during which the presented data was gathered, the natural process of aging, diseases, pests, pollution, climate changes, insufficient care and maintaining, has negatively impacted some of trees and shrubs used as seed sources in our investigation. This loss of potential seed sources is particularly adverse in the case of rare specimens from our investigation, such as *Ideasia polycarpa* Maxim. and *Cudrania tricuspidata* Bureau which are now unavailable for further propagation. During the on-field observations, we have registered dying of some other specimens (*Alnus cordata* Loisel., *Paulownia tomentosa*, *Libocedrus decurrens* Torr.), but in these cases the seed sources are generally not jeopardized yet. The forementioned factors have also impacted numerous other species, especially in residential green areas, but the reconstruction and emergence of new green areas have also introduced numerous other species. For example, since 2012, in the reconstructed Macedonia Park, *Ginkgo biloba*, *Larix decidua* Mill., *Metasequoia glyptostroboides* Hu&Cheng, *Picea pungens*, *Koeleruteria paniculata*, *Lagerstroemia indica*, *Liquidambar styraciflua*, *Liriodendron tulipifera*, *Platanus occidentalis* etc. are now present (Shotaroska et al. 2019), enlarging the existing seed sources from other parts of the city. Notably, these newly planted trees are mostly very young and therefore their fructification is still expected or insufficient, but they are to be considered as valuable seed sources, and regularly observed for their development progress. Thus, the urban areas of Skopje and the surroundings are promising as a seed source, but continuous management and observations are crucial for their maintenance. For example, the transformation of Skopje into a 'Green City' and the inclusion of a more varied type of green areas, e.g., green corridors and management of riparian areas (Penchikj and Hadzi Pecova 2018), might be a unique opportunity for on-field trials. From the investigated species that are of particular interest for ornamental use, those classified as 'rare' need to be treated as "endangered" and preserved by propagation. These seed sources cannot provide sufficient reproductive material for mass production, however, even the smallest quantity of seeds needs to be sown and seedlings distributed on various locations, to create new groups of trees and shrubs, which will fructify in the future. This is the case for species such as *Aesculus glabra* Willd. and *Melia azedarach*, which seem to be interesting for their potential for air pollution remediation, but are rare in Skopje and have exhibited low germination rates. On the contrary, species such as *Acer negundo*, *Fraxinus americana* and *Robinia pseudoacacia* have emerged as abundant and with medium to high germination rates (Table 1), and they need to be more closely monitored in Skopje due to their potentially negative impact.

Not all urbanized areas are used in the same manner and undergo same maintenance so the risk of spontaneous emergence of invasive species differs, which needs to be taken into consideration (Deparis et al. 2022). This is also the case in Skopje, where we have observed a variety of the urban landscapes (riverbanks, parks, residential areas of different range, industrial and semi-industrial zones etc.),

whose impact further complicates species selection and requires interdisciplinary expertise. The location where the species is planted has an impact on its functionality and can further enhance or diminish its services, since plants employ different trade-off and adaptation strategies in adverse conditions (Zhu et al. 2020). For example, *Liquidambar styraciflua* has shown to be efficient in rainfall and stormwater mitigation on vacant and underutilized urban land (Kirnbauer et al. 2013). In a study in Germany, Gillner et al. (2017) concluded that while species employ different strategies to deal with the heat and drought in urban areas, *Corylus* and *Tilia* were efficient even at stressful sites, while *Liriodendron tulipifera* and *Ginkgo biloba* were limited to the less demanding urban areas. *Prunus majestica* Koehne. was very efficient in PM accumulation in the industrial area, but *Osmanthus fragrans* Lour., *Loropetalum chinense* Oliv. and *Cinnamomum japonicum* Siebold. have shown to be more efficient in PM accumulation in high traffic and university campus area, indicating that the species efficiency varies depending on the level and type of air pollution (Li et al. 2019). With this in mind, we have identified some of the pressing requirements of the urban greenery and we have provided further details of the potential species suitability in Skopje based on reported experience in literature. One is the formation of the so-called urban heat island, the phenomenon of increased air temperatures in urban areas in comparison to their rural surroundings. The mitigation of the urban heat island does not depend only on the vegetation, but appropriate species selection has a positive impact (Ballinas and Barradas 2016). Generally, some species such as *Gleditsia triacanthos*, *Pyrus calleryana* 'Chanticleer' Decne., *Platanus × hispanica* 'Acerifolia' Mill. and *Acer campestre* L. have been noted as more tolerant to urban environmental stress, while, by comparison, *Tilia × europaea* 'Pallida', *Tilia cordata* 'Greenspire' Mill. and *Ginkgo biloba* have shown the lowest tolerance (Swoczyna et al. 2010). Urban water availability and management needs also to be taken into consideration for the species selection, since it can result in sustainable water costs and/or increased species mortality and low establishment success (Pataki et al. 2011). Furthermore, the water use efficiency can be a useful metric and one of the criteria for suitable selection of urban tree species, since species with low water use are not always characterized with reduced growth and the balance between the services the vegetation it provides, and the costs for its establishment could be optimized (McCarthy et al. 2011). The *Fraxinus* genus has been characterized with a wide genotypic variation in the drought response, with species such as *F. excelsior*, *F. nigra* Marshall., *F. ornus* L., and *F. angustifolia* Vahl. exhibiting higher and species such as *F. americana* and *F. velutina* Torr. exhibiting lower sensitivity to drought (Percival et al. 2006). One way to do a selection is by comparing the leaf gas exchange parameters as an indicator of drought tolerance, which in the *Magnolia* genus has pointed towards *Magnolia grandiflora* L. as a better alternative to *Magnolia × soulangeana* (Vastag et al. 2020). *Liquidambar styraciflua* is another species with noted ability to persevere seasonal drought and increased soil salinity (Baraldi et al. 2019b), as well as *Maclura pomifera* Schneid., which could more easily recover from the common drought cycles (Khaleghi et al. 2019). Trees could additionally reduce

the water run off during heavy rains in urban areas, and their efficiency in this again depends on the morphological characteristics, i.e., the leaf area, shape and size, in which smaller leaves are shown to be more efficient (e.g., *Ginkgo biloba*) (Yang et al. 2019). Species with smaller leaves are also able to stay cooler at higher air temperatures, e.g. *Gleditsia triacanthos* (Leuzinger et al. 2010). Another issue that has to be considered is the species susceptibility to pathogens, which some of the listed species have exhibited in other environments. For example, weakened *Pinus omorika* Pančić trees by drought and tree-pathogenic fungi have been threatened by the double spined bark beetle (*Ips duplicatus*) (Vakula et al. 2021). The great spruce bark beetle (*Dendroctonus micans*) has been determined as a new threat for *Picea pungens*, but it has also been shown to infect other species from the *Picea*, *Abies*, *Larix*, *Pinus* and *Pseudotsuga* genera (Lukášová et al. 2014). *Ginkgo biloba* has shown to have a high resistance to pathogens, as well as air pollution (Dmuchowski et al. 2019). An emerging area of interest is the carbon sequestration capacity of urban trees. Some species indeed have exhibited higher potential for it, such as *Prunus cerasifera* Ehrh., *Quercus cerris* Blanco., *Celtis australis*, *Acer campestre* and *Acer platanoides* (Baraldi et al. 2019a), while for some the urban residual biomass from maintenance could be used for biofuel production, e.g., *S. japonica* (Sajdak and Velazquez-Martí 2012). The literature revision has also revealed some specific issues (and potential research topics) regarding the vegetation in the urban and semi urban areas in Skopje that need to be addressed, i.e., better understanding of the tree stability in urban areas (Kontogianni et al. 2010, Göcke et al. 2018), the role of the urban fungi communities and associations (Karpati et al. 2011), the carbon sequestration potential in urban soils (Schmitt-Harsh et al. 2013), the plants' phyllosphere bacteria interaction for the reduction of air pollution (Franzetti et al. 2020), landfill revegetation and regeneration (Kim and Lee 2005), urban plant biomass use for compost and biofuel production (Ljubojević et al. 2021) etc. Considering the numerous factors that impact tree development in urban areas, as well as the services they are expected to provide, species assessment process through multi-criteria decision-making methods (Ghafari et al. 2020), dendroecological studies with Climate-Species Matrix (Roloff et al. 2009) or the use species selection models (Li et al. 2011) could be of interest for future interdisciplinary research.

Beyond urban forestry, the use of allochthonous woody plants for forestry purposes (afforestation and reforestation) is not a novelty, and it is a well-known strategy especially in the context of climate adaptive forestry (Bolte et al. 2009, Brus et al. 2019). Urban areas can be used for case studies of the climatic change effects on forests, as part of urban forests which are already exposed to conditions expected to occur in (semi) natural forests in the coming future, e.g., increased soil content of nitrogen, elevated CO₂ level, increased temperature on the urban heat islands etc. (O'Brien et al. 2012). Previous reforestation efforts in MKD have included some of the analysed species, e.g. *Cupressus arizonica*, *Acer negundo*, *Fraxinus americana*, *Robinia pseudoacacia*. These species are also abundant in the green areas of Skopje, require less demanding pre-sowing treatment and exhibit high germination rate and good seedling establishment

(Supplementary Material 1), but as previously mentioned, they have been known for their invasive potential. This reduction of species diversification in afforestation urgently needs to be addressed as erosion rates in the rural and mountainous areas are rising under the climatic pressure and natural field conditions (Kolevska et al. 2017). Thus, urban allochthonous species can serve as a seed source (Zhu and Lou 2013) and living experiments to better understand the stages of plant establishment and optimization of the sowing and growing protocols. Considering the climatic and ecological conditions, as promising species we underline *Cryptomeria japonica*, *Alnus cordata*, *Carya pecan*, *Juglans mandshurica* Maxim., *Quercus aegylops* L. *Cedrus deodara*, *Cedrus atlantica*, *Ginkgo biloba*, *Larix decidua*, *Ulmus pumila*, *Juglans nigra*, and *Sophora japonica*, some of which have been previously used in small-scale reforestation efforts (Kolevska, personal communication), but further studies and trials are needed.

CONCLUSIONS

Urban areas continue to be under significant pressure due to rural-urban migrations and land availability leading to various types of (urban) pollution, e.g., air, noise, water etc. This, in combination with extreme temperatures and climatic changes severely threatens the life quality in urban regions. Urban forestry and green infrastructures, primarily increasing the green public spaces, is a promising solution (Arsovski et al. 2018), yet species selection, appropriate planning and maintenance are crucial factors for success. In the city of Skopje and its surroundings, green areas are comprised of both autochthonous and allochthonous species, the later ones being of particular interest in the past century. In attempts to contribute to the limited body of knowledge, the present study summarises 25 years of experience with allochthonous woody plants from the Skopje region, focusing on their presence and generative propagation potential. This information, combined with

literature revision, has allowed us to pinpoint the allochthonous woody species of potential benefit and risk for Skopje. Furthermore, it has allowed us to conclude that indeed tracking the allochthonous species from urban areas is essential for insuring seeds and seedlings of higher quality and establishment success (Babapour et al. 2020). An accompanying factor are the nursery production systems (Allen et al. 2017) which, as emphasised in previous studies, need to be improved, modernized and better-adapted (Kolevska et al. 2017). Future, long-term studies and interdisciplinary approaches that consider the biological characteristics of the species under the changing climatic conditions, would allow for better overview and more solid recommendations for practical use.

Author Contributions

AD and DDK conceived and designed the research. DDK collected the plant material, performed the laboratory analysis, provided the tables data, supervised the research and provided valuable feedback to the manuscript. AD and VBS wrote the manuscript and jointly contributed to the figures. AD dealt with the revision process.

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Conflicts of Interest

The authors declare no conflict of interest.

Supplementary Materials

Supplementary File 1 - Additional information regarding the presence and generative properties of the examined species. Data for the examined woody plants (total of 65) including: Species Latin name, level of presence in Skopje, Germination or viability, type of pre-sowing treatment applied, 1+0 old Seedlings features (height and root collar diameter).

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Regeneration Analysis of the *Juniperus excelsa* Mixed Stands in Prespa National Park of Greece as a Base for the Assessment of the Appropriate Silvicultural Treatment for the Conservation of the Species

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ABSTRACT

Analysis of the regeneration of mixed stands of *Juniperus excelsa* (Greek juniper) in Prespa National Park revealed two distinct structural types: a) stands with small gaps, and b) stands without gaps. Fifteen 500-square-meter sample plots were established in each structural type. All plant species were counted in each plot, and Greek juniper plants were classified into two groups based on their regeneration status. Plants that have been established and grow under the facilitation of other plants fall into the first group, while those that have been established and grow in light, in canopy gaps, belong to the second. Regarding the regeneration of Greek juniper in the Greek juniper mixed stands with small gaps, facilitation is not the primary mechanism at work. There are less Greek juniper regeneration plants in the gap-free structural type, compared with the small-gap type. Greek juniper regeneration plant density will decrease if gaps close. Finally, in both structural types, the other species' regeneration plants exhibit higher density than those of Greek juniper. Creating gaps around Greek juniper trees by extensive intervention is one of the most successful ways for the forest practice to protect the Greek juniper mixed stands.

Keywords: gaps; facilitation; Greek juniper; Prespa National Park; silvicultural intervention

INTRODUCTION

Juniperus excelsa M. Bieb. (Greek juniper) is a species that can germinate and grow in direct sunlight as well as establish and survive in shade for decades (Milios et al. 2007, Milios et al. 2009). It can survive and grow under various disturbance and competition regimes, while it can exhibit the highest height growth at a wide range of ages and it can have significant increases in growth of ring width, even at an old age (Milios et al. 2009). Moreover, Milios et al. (2009) refer that Greek juniper can exhibit high rates of height increment in productive sites. Greek juniper can be planted in degraded areas in the context of restoration programs (Stampoulidis et al. 2013), since it can grow and usually is found in areas with harsh and severe abiotic conditions, as

well as in grazed, disturbed and degraded sites (Hall 1984, Ahmed et al. 1989, 1990, Fisher and Gardner 1995, Gardner and Fisher 1996, Carus 2004, Milios et al. 2007, 2009, 2011, Ozkan et al. 2010, Stampoulidis et al. 2013). According to Stampoulidis et al. (2013), in the future, Greek juniper could be introduced in areas in which the regeneration of native species will be difficult, due to unfavorable climatic conditions, which are a result of climate change.

Southeastern Balkans, Crimea, Anatolia, western Asia, and Arabic peninsula are all part of the species' range (Douaihy et al. 2013, Tavankar 2015, Korakis 2015, Mazur et al. 2021, Yücedağ et al. 2021).

The mountains of Macedonia and Thrace, as well as several Greek islands, are habitat to this species in Greece (Milios et al. 2007, Stampoulidis et al. 2013, Korakis 2015).

Greek juniper is protected by the Greek law and its forests belong to a habitat type that is comprised into the European Habitat Directive 92/43 and characterized as a priority habitat type (Korakis 2015). It is found as a species in degraded scrublands, as solitary trees, in small groups mostly on rocky slopes, and only very rarely in larger formations of pure or mixed stands (Milios et al. 2007). Prespa National Park, located in the northwest of Greece, is the primary region where Greek juniper creates massive formations (Stampoulidis et al. 2013). Restoration activities are required for the conservation of the formations of the species in the Prespa National Park (Kakourous and Fotiadis 2014).

In the Prespa National Park there are pure and mixed Greek juniper stands. In the pure stands Stampoulidis and Milios (2010) distinguished two site types: the first type represents good sites qualities (productive sites) and the other type represents medium sites qualities (less productive sites). In both site types there are dense and sparse pure groups and stands of the species. In pure Greek juniper stands, two tree forms, regarding their foliage, have been observed. In the first form, the living foliage starts from the ground level, while in the second form the living foliage appears from a height of 50–60 cm above the ground level (Stampoulidis and Milios 2010). In Greek juniper forests of the Prespa National Park, the forest service does not apply silvicultural treatments (Kakourous and Fotiadis 2014). However, silvicultural treatments have to be applied for the long-term conservation of the species in the area.

Apart from Prespa National Park, another area where Greek juniper creates formations is in the middle portion of Nestos Valley in northeastern Greece (Milios et al. 2007). Stampoulidis (2010) pointed out that the maximum height of Greek juniper trees is higher in the Prespa National Park, compared to that of the formation of the species in the middle portion of Nestos Valley, while in Prespa National Park the density of Greek juniper trees ($h > 1.30$ m) is lower than that of the mixed formations of the species in the middle portion of Nestos Valley.

Analysis of the ecosystems of the target species is necessary for the creation of suitable silvicultural treatments aimed at the conservation of tree species that are under threat or significant for any reason. Stand structure assessments and regeneration analyses are two crucial parts of this analysis (Milios 2021, Milios et al. 2021).

The goal of this research is to use findings from a regeneration analysis of Greek juniper mixed stands in Prespa National Park, Greece, to determine the best silvicultural approach for the long-term survival of the species.

MATERIALS AND METHODS

Study Area

The study was conducted in the western part of the Prespa National Park in northwestern Greece (40°49'12.88"N, 21°2'31.98"E), covering an area of about

2,732 ha and ranging in elevation from about 840 to about 1,360 meters. There are both pure and mixed stands of Greek juniper in this region.

Greek juniper may be seen growing in groups or as solitary trees in mixed stands. This study's sample plots were located in an area dominated by *Quercus macedonica* DC. or *Quercus pubescens* Willd., with *J. excelsa*, *Carpinus orientalis* Mill., and *Acer monspessulanum* L. serving as co-dominant species in the overstory. Furthermore, *Juniperus oxycedrus* L. species occurs mostly in a shrubby form in mixed stands.

The soils are clay to clay-silts, while the substratum consists of limestones and dolomitic limestones (Pavlides 1985). At least one soil profile was taken in each established plot to measure soil depth in Greek juniper mixed stands. The soil depth is between 25 and 40 centimeters deep.

The average annual rainfall is 817 mm, and the average temperature is 10.8°C, according to data from the Nestorio meteorological station (which is adjacent to the region).

Field Measurements

There is little variation in site productivity across plots due to the small variation in soil depth found in the established plots. However, the percentage of the stand area covered by the tree canopy projection (max=100%) was not constant. Two distinct structural types were identified according to the estimated canopy cover percentage of a stand (estimated area covered by the tree canopy projection of a stand \times 100/area of a stand):

a) Canopy cover percentages in MSSG (Mixed Stands with Small Gaps) ranging from 65% to 75% (Figure 1).

b) Canopy cover percentages in MSWG (Mixed Stands Without Gaps) ranging from 95% to 100% (Figure 2).

The density of Greek juniper trees is 135 per hectare in the MSSG structural type and the total number of trees of all species (Greek juniper included) is 441 per hectare, whereas the corresponding values in the MSWG structural type are 144 per hectare and 664 per hectare (a multi-stemmed tree was counted as one tree) (Stampoulidis 2010). In MSSG structural type the mean height is 6.17 m with standard deviation of 2.372 m for Greek juniper, and 4.62 m with standard deviation of 1.783 m for the other species. In MSWG structural type the mean height is 6.95 m with standard deviation of 2.314 m for Greek juniper, and 6.11 m with standard deviation of 2.015 m for the other species (Stampoulidis 2010).

Thirty identical 500-square-meter sample plots (20 m \times 25 m) were set at random in the summer of 2009. Fifteen sample plots were established in MSSG and fifteen sample plots in MSWG. The established plots exhibited the canopy cover percentage range of their structural type. The slope varied from 10% to 30% across all plots with various exposures across all plots. Each plot was surveyed for all regeneration plants and tallied for seedlings (plants up to 1.3 m in height), and Greek juniper regeneration plants were classified into two types. Greek juniper regeneration, which has been established and grows under the facilitation of other plants, is the first type. Regeneration found under closed canopy or at the edge of the canopy (up to 30 cm out of the projection of the canopy edge) of a single plant or a group of plants of Greek juniper or other species, falls into

this type. The second type represents the regeneration that has been established and grows under light, in small gaps (Stampoulidis et al. 2013).

Statistical Analysis

The non-parametric Mann-Whitney and Wilcoxon tests were used to assess the density data from the regeneration process. Since the assumption of normality of data distributions was not met, GLM analysis was not performed on the data. SPSS was used for all statistical analyses (IBM 2021).

RESULTS

When comparing the number of Greek juniper regeneration plants exposed to facilitation vs the number of plants exposed to light in gaps of the MSSG structural type, there is no statistically significant difference (Table 1). Regeneration plants are more numerous ($p < 0.05$) in the MSSG structural type than in the MSWG structural type (Table 2). In addition, other species exhibit more regeneration plants ($p < 0.05$) than Greek juniper in both structural types of mixed stands (Table 2).

The number of regeneration plants per hectare in the two Greek juniper mixed stand structural types are shown in Figure 3. No Greek juniper regeneration plants could be seen growing in the light in the MSWG structural type. The following taxa are represented by the regenerating plants of other species found in the two structural types:

MSSG: *Quercus pubescens*, *Quercus macedonica*, *Carpinus orientalis*, *Fraxinus ornus* L., *Cornus mas* L., *Acer monspessulanum* and *Juniperus oxycedrus*.

MSWG: *Quercus pubescens*, *Acer monspessulanum*, *Quercus macedonica*, *Carpinus orientalis*, *Juniperus oxycedrus*, *Quercus cerris* L. and *Cornus mas*.



Figure 1. MSSG (Mixed Stands with Small Gaps). In the foreground of the photograph, a Greek juniper regeneration plant can be seen. The rest of the trees in the photograph are other tree species.



Figure 2. MSWG (Mixed Stands Without Gaps). On the left side of the photograph a part of trunk of a Greek juniper tree can be seen, while in the background on the right side of the photograph a Greek juniper tree can be faintly seen. The rest of the trees in the photograph are other tree species that grow in mixed stands.

Table 1. Density of *Juniperus excelsa* regeneration plants in the two structural types of *J. excelsa* mixed stands: MSSG (Mixed Stands with Small Gaps) and MSWG (Mixed Stands Without Gaps).

Structural type	Number of <i>J. excelsa</i> regeneration plants in light in small gaps				Number of <i>J. excelsa</i> regeneration plants under facilitation				Number of sample plots
	Mean	SD	Max	Min	Mean	SD	Max	Min	
MSSG	0.80a	0.676	2	0	1.07a	1.624	5	0	15
MSWG	0	0	0	0	0.73	1.100	4	0	15

In MSSG structural type, the means in a line are statistically significant different at $p < 0.05$ when they share no common letter. The comparison was made using the Wilcoxon test, SD=Standard Deviation

Table 2. Density of regeneration plants in the two structural types of *J. excelsa* mixed stands: MSSG (Mixed Stands with Small Gaps) and MSWG (Mixed Stands Without Gaps).

Structural type	Total number of <i>J. excelsa</i> regeneration plants				Total number of other species regeneration plants				Number of sample plots
	Mean	SD	Max	Min	Mean	SD	Max	Min	
MSSG	1.87ab	1.767	6	0	64.80a	20.557	107	31	15
MSWG	0.73bb	1.100	4	0	72.53a	38.643	147	18	15

Juniperus excelsa means in the column are statistically significant different at $p < 0.05$ when they share no common letter in normal print. The comparison of the total number of *J. excelsa* regeneration plants between the two structural types was made using the Mann-Whitney test. Means in a line are statistically significant different at $p < 0.05$ when they share no common letter in bold print. The comparisons between *J. excelsa* and other species' regeneration plants in both structural types were made using the Wilcoxon test, SD=Standard Deviation.

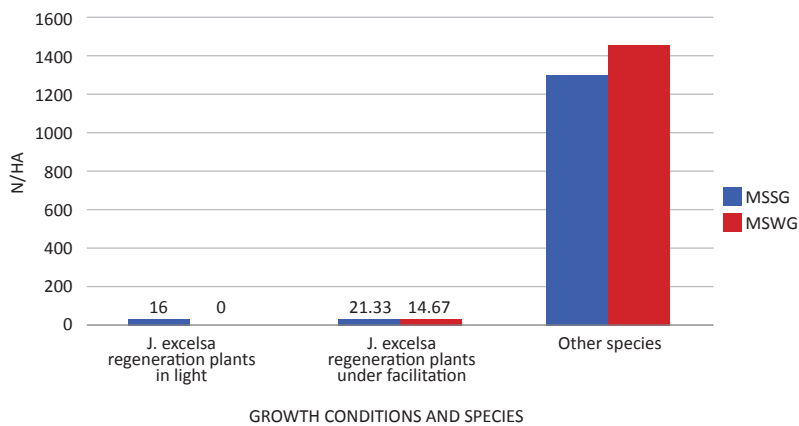


Figure 3. Density per hectare of *J. excelsa* (in light and under facilitation) and other species regeneration plants in the two structural types of mixed stands: MSSG (Mixed Stands with Small Gaps) and MSWG (Mixed Stands Without Gaps).

DISCUSSION

Based on the results of this study, Greek juniper can be established in both light (in small gaps) and shade (under canopy), the same as the literature refers (Milios et al. 2007, 2009, 2011, Stampoulidis et al. 2013). It is not surprising that Greek juniper regeneration plants could not be located in light in the MSWG structural type, since there was no place for them to grow and develop in gaps while receiving full light.

No significant difference was found between the number of plants established and growing under facilitation and those established and growing in full light ($p>0.05$), suggesting that facilitation is not the major mechanism in the regeneration of Greek juniper in mixed stands with small gaps (MSSG). To a large extent, facilitation may affect the success of plant establishment (Badano et al. 2016, O'Brien et al. 2019, Collins et al. 2019, Lucero et al. 2019, Petrou and Milios 2020, Duarte et al. 2021). When discussing the importance of nursing plants in the establishment of Greek juniper plants, Milios et al. (2007) note that in the middle portion of Nestos Valley in Greece, almost all of the regeneration plants of the species were discovered under the facilitation of nurse plants. There are regeneration plants of the species that were established under facilitation in Cyprus, although this process is not the dominant in the regeneration of the species (Milios et al. 2011). Furthermore, Stampoulidis et al. (2013) in pure Greek juniper stands in Prespa National Park discovered that facilitation does not dominate as a process in the regeneration of Greek juniper; however, it is important as a process, since a sizable proportion of regeneration plants were established and grew under facilitation. So, in restoration programs tree seedlings can be established under the facilitation of a group of trees or individual trees (Milios et al. 2007, 2009, 2011, Stampoulidis et al. 2013).

The fact that the structural type without gaps (MSWG) shows a lower ($p<0.05$) number of Greek juniper regeneration plants than that with small gaps (MSSG),

clearly points out that if the gaps close, the density of Greek juniper plants regeneration will decrease. It is worth noting that in MSWG, there were only 14.67, Greek juniper regeneration plants per hectare (Figure 3). On the other hand, in both structural types, the regeneration plants of the other species show higher density ($p<0.05$) than those of Greek juniper (Table 2) and exhibit a very high number of plants (Figure 3).

Regeneration of broadleaf species is being intensively grazed in the study region. Grazing is therefore one of the primary disturbance sources (Stampoulidis et al. 2013). Vegetation composition in mixed stands will shift, and it is likely that Greek juniper individuals may become rare if grazing is not intensive (or ceases) and does not prevent the entire dominance of broadleaf species. Greek juniper is a site insensitive species that is supplanted by more site sensitive species at better sites, when disturbances cease (Milios et al. 2007, Milios et al. 2011). Reducing human disturbance (mostly grazing), and the establishment of other species, are two key factors threatening the survival of *Juniperus thurifera* L. in deep-soil regions of the Pyrenees and the Alps, as stated by Gauquelin et al. (1999).

Effective steps should be taken by the forest practice to protect the Greek juniper mixed stands in Prespa National Park. The establishment of Greek juniper regeneration plants will be promoted by creating gaps around Greek juniper trees by extensive interventions and then controlling the spouts of other species, as well as the regeneration of competing species in the gaps. As a result, Greek juniper plants will be established without facing competition from other species, leading to a greater presence of this species in the mixed stands.

Removing individuals of potentially competitive species will help the Greek juniper regeneration plants in the future. For the stands in central part of the Nestos Valley in Greece and for the stands of the species in Cyprus, Milios et al. (2007 and 2011) recommend the similar technique of releasing Greek juniper plants from the competition of other species.

CONCLUSIONS

There are less Greek juniper regeneration plants in the gap-free structural type, compared with the small-gap type. Greek juniper regeneration plant density will decrease if gaps close. In both structural types, the other species' regeneration plants exhibit higher density than those of Greek juniper. Creating gaps around Greek juniper trees by extensive intervention is one of the most successful ways for the forest practice to protect the Greek juniper mixed stands. Removing individuals of potentially competitive species will help the Greek juniper regeneration plants in the future.

Author Contributions

AS and KK conceived and designed the research, AS carried out the field measurements, AS, KK, EP and PP processed the data and performed the statistical analysis, AS, KK, EP and PP wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest

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Logging Residue Assessment in Salvage Logging Areas: a Case Study in the North-Eastern Italian Alps

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ABSTRACT

Salvage logging operations often occur after large disturbances and usually leave behind a substantial quantity of residues, which is fundamental for maintaining soil fertility and facilitating ecosystem dynamics. This study aims to estimate the amount of logging residues following salvage operations categorized by two wood harvesting systems: Cut-To-Length (CTL) and Full-Tree System (FT). Logging residues in the harvested areas were sampled using linear transects and the data collected were divided into classes based on diameter. The quantity of residues was estimated using the Brown method for Fine Wood Debris (FWD) and the Van Wagner method for Coarse Wood Debris (CWD). Furthermore, the carbon and nutrient content associated with logging residues were also determined, considering their interaction with the soil organic layer. Overall, a higher quantity of FWD was detected in the sites cleared with the FT system and a higher quantity of CWD in the sites logged with the CTL system. Differences could be observed for all three years and systems considered, but only the third year reported statistically significant results ($p < 0.01$). The soil and residue chemical analysis for carbon and nutrient contents revealed a high amount of carbon stored in a potential layer of 10 cm of soil (up to 85 Mg·C·ha⁻¹), while only up to 15 Mg·C·ha⁻¹ for the woody material.

Keywords: forest mechanization; carbon cycle; impact; management; sustainability

INTRODUCTION

In the past few decades, extreme climatic events, such as wildfires and windstorms, have increased their incidence in Europe (European Environment Agency 2019, Seidl et al. 2017) with an annual average of 0.52 disturbance patches per square kilometres of forest area (Senf and Seidl 2020). This situation made European forests more vulnerable and prone to extensive damage, with fires occurring mainly in the Mediterranean region (Verkerk et al. 2018) and storms in Northern and Central-Eastern Europe (Forzieri et al. 2019). In recent years, however, storms have become a relevant issue even in Southern Europe (Cavaleri et al. 2019, Forzieri et al. 2019, Pilli et al. 2021).

Forests are characterized by high multifunctionality, delivering multiple ecosystem services (Brocknerhoff et al. 2017), especially in mountain areas where they provide not only timber production but also land protection, water supply and recreation (Häyhä et al. 2015). Furthermore, forest

stands can capture and store carbon, with half of the global organic carbon in terrestrial ecosystems being stored in forest soils (Mayer et al. 2020). Moreover, European forests in the last decade have sequestered each year more than 155 Mt of carbon (Forest Europe 2020). In this way, carbon gets stocked in trees and surrounding soil, where it builds up nutrients (Bauer et al. 2000). However, this important function is put at risk by climate change and the increasing incidence of extreme events (Lindner et al. 2010). To preserve this storage function and to mitigate climate change-induced events, alternative strategies are proposed and adapted to local needs and requirements (Irauschek et al. 2017, Kramer et al. 2014, Mina et al. 2017, Pilli et al. 2021, Priewasser et al. 2013, Wohlgemuth et al. 2017).

Consequently, due to the increased incidence and magnitude of extreme events, the increase of damaged forest areas leads to an increase in salvage logging operations. Salvage logging is defined as extracting damaged timber from a disturbed forest area to minimize the economic loss

of forest stands (Lindenmayer 2006). These operations are complex and pose an elevated risk of accidents. When damages occur in small and easily accessible areas close to forest roads, traditional semi-mechanized configuration systems, generally based on motor-manual processing and extraction by tractor and winch or by forest skidders (Borz et al. 2013, 2014, Iranparast Bodaghi et al. 2018), represent a valid approach despite elevated risk of accidents for the operators (Sanginés de Cárcer et al. 2021). In the case of large and damaged areas, the operations are generally performed by fully or highly mechanized configurations in order to i) foster an immediate removal of damaged trees, ii) maintain high efficiency in terms of productivity and iii) reduce logging and transportation costs (Heinimann et al. 2006, Iranparast Bodaghi et al. 2018), ensuring a higher safety level at the same time (Kymäläinen et al. 2021, Sanginés de Cárcer et al. 2021).

Salvage logging operations usually have high environmental impacts in comparison to ordinary operations, threatening ecosystem biodiversity (Thorn et al. 2018), disrupting services provided by the forests (Leverkus et al. 2018), amplifying the risks of impact on damaged forest areas in terms of soil exposure and risk of erosion (Prats et al. 2021, Robichaud et al. 2020), risk of soil degradation, with the loss of nutrients and carbon and therefore loss of fertility (Valipour et al. 2021), as well the risk of biodiversity loss (Thorn et al. 2018).

Salvage logging also affects the biomass left after the operations, such as branches, tops and stumps, hereafter referred to as “residues”. The quantity and quality of residues left (fine or coarse woody debris) depend on the harvesting methods adopted (Huber et al. 2017, Tamminen et al. 2012): in salvage logging operations both Cut-To-Length (CTL) and Full-Tree (FT) extraction systems can be adopted (Figure 1a and Figure 1b).

In salvage logging operations, when the terrain is gentle, CTL is often associated with fully mechanized systems, with

combined use of a harvester and a forwarder. Adopting the fully mechanized CTL system increases the level of safety in salvage logging operations with consequent reduction of the risk of accidents for the operators (Cadei et al. 2020, Sanginés de Cárcer et al. 2021). In the case of fully mechanized CTL, the tree is processed at the stump site by the harvester machine and consequently most of the logging residues are left on the ground homogeneously distributed or piled in heaps (Nurminen et al. 2006).

When the terrain gets steeper, cable yarding is the preferred system, viable also considering salvage logging conditions (Spinelli et al. 2022), favouring a higher mechanization level and efficient processing of the full tree at roadside by using excavator-based processor head or by using a processor mounted on a cable tower yarder (Mologni et al. 2016). In this case, the entire tree is extracted with the branches and the top, delimbed and cut by the processor head, thus accumulating most of the logging residues at the roadside, in the same area in which the trees are processed. Therefore, the full-mechanized CTL system and the FT systems represent two opposite ways of logging in terms of residue generation and removal from the forest.

Specifically for the two machine configurations, cable yarding is considered to have smaller impact than ground-based mechanized system (Mologni et al. 2016, Stanturf 1990). In fact, cable yarding extraction has lower impact on soil compared to ground-based systems (e.g., harvester and forwarder or tractor and winch system) (Krag et al. 1986, Lafan et al. 2001, Miller and Sirois 1986) and lower costs at the same time (Heinimann et al. 2006). However, in terms of residues left in the forest (e.g., branches, top and stump), conventional FT harvesting has a greater amount of nutrients removed from harvest sites than in CTL harvesting due to the extraction of nutrient-rich branches and foliage (Huber et al. 2017).

Forest residues and deadwood are major sources of nutrients and carbon (Janowiak and Webster 2010, Palviainen



Figure 1. Effect of the application of different harvesting systems and machine configuration for salvage logging operations in southern Alps after the Vaia storm in 2018 based on (a) a CTL system with fully mechanized harvesting system with the integration of harvester and forwarder machines, and (b) based on a FT system with semi-mechanized felling, cable yarder extraction and tree processing at roadside.

et al. 2010). Variations in quantity and quality of residues affect soil fertility (Mayer et al. 2020), putting at risk site regeneration and future growth of forests (Bačič et al. 2012, Bauer et al. 2000, Motta et al. 2006, Zielonka and Niklasson 2001). Furthermore, forest biodiversity can also be affected by disturbance regimes such forest fires and bark beetle outbreaks (Carlson et al. 2017, Mattson et al. 2019, Sullivan et al. 2021). Moreover, they create uncertainty in carbon stock quantification since fine residues are usually ignored in the estimations (Maas et al. 2020). Also, there is an increasing interest in the retrieval of this material for bioenergy production (Bessaad et al. 2021).

The aim of this study is thus to verify and quantify the effect on logging residues' quantity due to the adoption of different harvesting system and configuration in salvage logging operations, and consequently estimate the difference in terms of carbon and nutrient potential of residual availability for *in situ* nutrient and carbon cycle. Moreover, we also want to investigate the timing of operations and how this affects the residues' quantity.

MATERIALS AND METHODS

Study Area

The mountain forests considered (S. Martino di Castrozza, Paneveggio e Cadino) are located in the province of Trento in

north-eastern Italy and belong to the Autonomous Province of Trento (Figure 2). The forests consist of a typical spruce mountain forest, mainly composed of Norway spruce (*Picea abies* (L.) H. Karst) and larch (*Larix decidua* Mill.), with an average altitude between 1,550–1,670 m a.s.l., and an average growing stock of 425 m³·ha⁻¹. These areas were all impacted by the Vaia storm at the end of October 2018.

Two sites having similar characteristics were selected from each forest stand: large and cleared forest areas, with the possibility to find forest machine tracks and comparable forest types. The sites from Cadino have east and north-west aspect, while all the other four sites face south or south-east. The sites have been all subjected to salvage logging in the period of 2019–2021 following the Vaia storm, with the use of both harvesting CTL systems, with harvester and forwarder (HF), and harvesting FT systems, using cable yarder (CY). The field sampling over the area was performed in the summer of 2021. Table 1 summarizes the main data related to the selected study sites.

The choice of the study areas is key for understanding this study approach: the similar conditions of the forests and the selected sites (i.e., in terms of forest type, growing stock, and aspect) are the main assumptions to shift the analysis from the geographical to the temporal scale. Each site will then represent the year in which it was harvested with respect to the system and configuration adopted during salvage logging operations to better evaluate the timing effect on residue quantities.

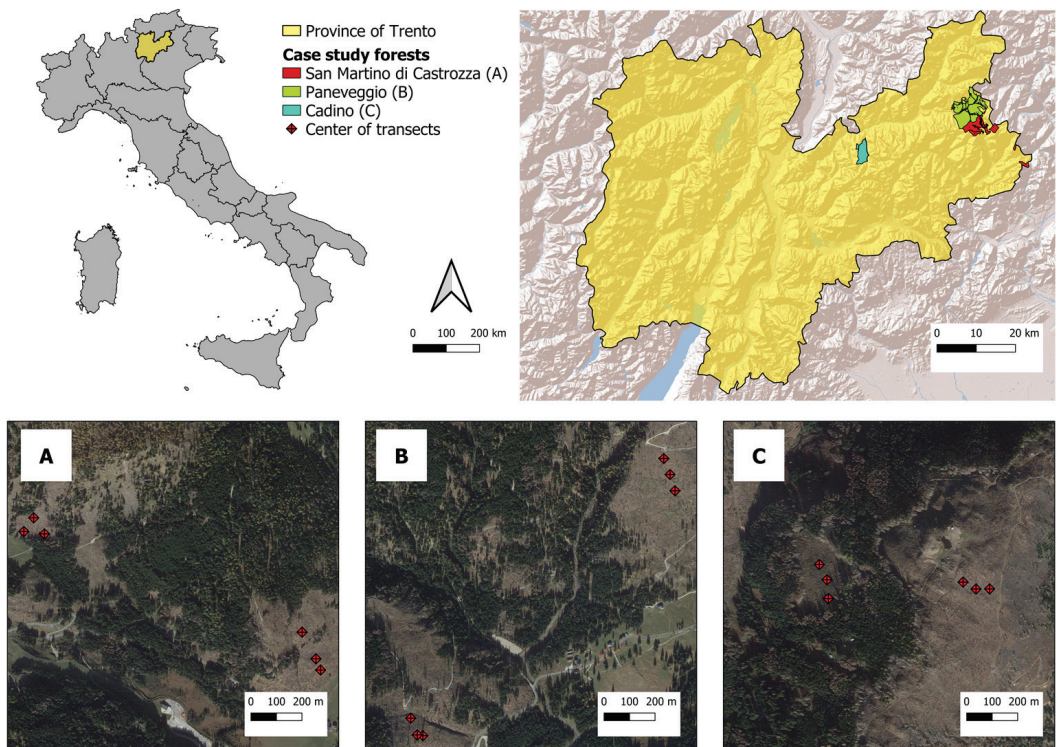


Figure 2. Study areas' locations.

Table 1. Summary of data related to the study sites selected. “Salvage logging” refers to the year of operation and the system adopted (HF - harvester-forwarder, CY - cable yarder); in brackets the years after the Vaia storm are reported.

	S. Martino di Castrozza		Paneveggio		Cadino	
Average altitude	1,570	1,670	1,650	1,550	1,660	1,640
Aspect	SE	S	S	SE	E	NW
Surface (ha)	14.48	19.92	24.28	18.38	16.88	20.12
Damaged surface (%)	92.5	27.5	69.7	63.5	96.3	19.1
Growing stock (m ³ ·ha ⁻¹)	456.13	321.15	442.51	517.97	399.58	414.33
Salvage logging	CY 2019 (1)	CY 2021 (3)	HF 2019 (1)	CY 2020 (2)	HF 2020 (2)	HF 2021 (3)
Site coordinates (EPSG 32632 coordinate)	714443 E 5127902 N	715537 E 5127363 N	711231 E 5132502 N	710260 E 5131493 N	684821 E 5122355 N	684824 E 5122355 N

Field Sampling and Target Material

The selected study sites have been investigated first using aerial photos to compare the situation before and after the Vaia storm. What is more, a canopy height model (CHM) was obtained to assess the size and distribution of the trees in the sites, therefore providing an initial understanding about the potential location of residues on site (Figure 3). The tree density and growing stock were lower in HF 2020 and CY 2021 than in the other sites, whereas the tallest trees, of more than 30 m, were found in HF 2021 and CY 2020. Lower stand density, with a higher gap fraction in the canopy cover, might suggest the presence of a higher crown ratio, and therefore the presence of more branches. In contrast, the presence of tall trees might indicate the presence of thinner material on site.

Field sampling of logging residues was performed by adopting a line intersect sampling (LIS) method, which estimates weights and volumes of down woody material (Brown

1974, Woodall and Monleon 2008) over completely clear-felled areas. The main assumptions when using this sampling technique are the random orientation of the woody debris under the linear transect, lying horizontally, having a circular shape, and a normal distribution within diameter classes. For this study, the sampling method (Figure 4) was initially adapted from Rizzolo (2016), registering the diameter of each woody debris under a 20 m line and classifying them in time lag classes (Table 2). This division refers to the time required for a fuel particle to change its moisture content accordingly to the equilibrium moisture content but can be easily adapted to other applications: material finer than 76 mm in diameter belongs to fine woody debris (FWD) and larger material to coarse woody debris (CWD). The length has also been recorded for CWD larger than 203 mm.

Each sampling is composed of three parallel transects, the central one located where the forest machines (in the case of HF) or the cable line (in the case of CY) was passing

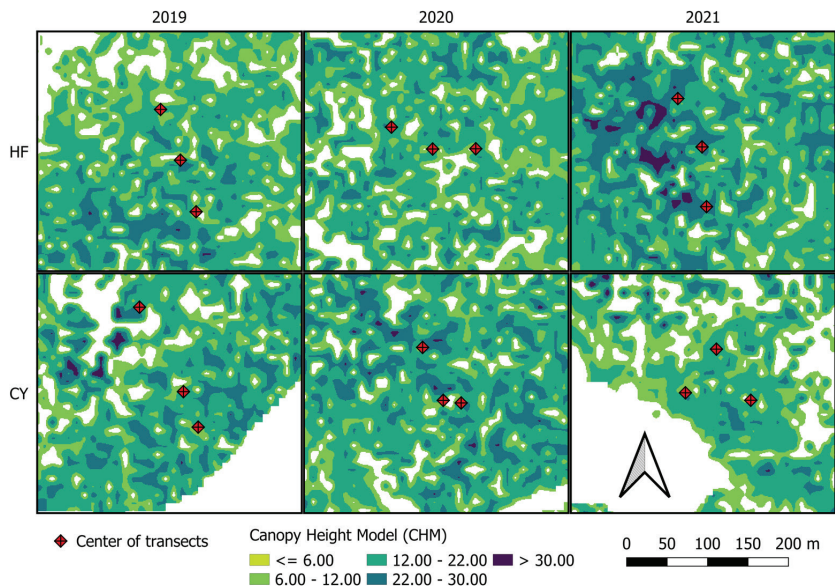


Figure 3. Canopy Height Model (CHM) of the considered sites with the centre of the transects.

and the other two on each side. The central one has been modified into a series of four sub-transects orthogonal to the extraction line to better account for the variability of residues and the harvesting system effect. The distance between the central transect and the lateral ones is 10 m for HF and 15 m for CY. Three samplings have been selected for each of the extraction lines, with a ground distance of 50 m between them, when possible. Moreover, to minimize road influence, the starting points were placed at least 20 m from the roadside for both HF and CY sites.

Residue Mass Estimation and Statistical Analysis

The residue quantity estimation ($\text{Mg} \cdot \text{ha}^{-1}$) was performed for both categories of residues: for FWD classes, the Brown's formula was used (Brown 1974), described in Equation 1. For bigger elements, CWD in this case, for Class D, a simplified version of Brown's formula was applied, shown in Equation 2. For Class E elements, instead, the estimator was computed using Van Wagner formula (Van Wagner, 1968), expressed by Equation 3.

$$\hat{Y}_{FWD} = \left(\frac{1.234 \cdot n \cdot \bar{d}^2 \cdot SG \cdot c \cdot a}{\sum L} \right) \cdot k_{decay} \cdot 10,000 \quad (1)$$

$$\hat{Y}_{CWD} = \left(\frac{1.234 \cdot n \cdot \bar{d}^2 \cdot SG \cdot c}{\sum L} \right) \cdot k_{decay} \cdot 10,000 \quad (2)$$

$$\hat{Y}_{CWD(E)} = \frac{\pi^2 \sum d^2}{8L} \quad (3)$$

Where: 1.234 is a conversion constant derived from the literature; n is the number of elements for each class; \bar{d} is the average squared diameter for the class; SG is the specific gravity for the wood species considered; c is the corrected slope; a is the correction coefficient for the position of the elements, equal to 1.13 for FWD and equal to 1 for CWD; L is the length of the sampling line(s); k_{decay} is the decay coefficient as described by Woodall et al. (Woodall and Monleon 2008); 10,000 are the square meters in 1 ha. Due to the characteristics of the wood, as timber and debris were preserved over the previous two years by snow without major discoloration and with intact wood texture, an average SG of 0.44 was selected, with a density (ρ_w) of $0.44 \text{ g} \cdot \text{cm}^{-3}$ (oven dried). Similarly, the decay

of the material was considered as class 1 (Petrillo et al. 2016). The corrected slope was calculated as described in Equation 4.

$$c = \sqrt{1 + \left(\frac{\text{Slope}_{\%}}{100} \right)^2} \quad (4)$$

Additionally, the average value of residue mass was divided to analyse the spatial distribution between the logging trails (i.e., the central transect) and the surrounding areas (i.e., the lateral transects), as shown in Figure 4.

To better analyse the results, a normality test was performed using the Shapiro-Wilk test, as well as a visual assessment of the data distribution since the number of elements for each class-harvesting system-year was small. The result of the normality assessment was a non-normal distribution. In order to validate the residue estimations resulting from the same year, but performed with a different harvesting system, a statistical analysis was performed by comparing the estimators, in order to obtain any significant differences using the two-tailed Mann-Whitney U-test ($p < 0.01$).

Chemical Analysis of Soil and Residues

For each forest site, samples of soil and residues have also been collected for chemical analysis. Due to the large variability in forest soils, for each site, soil samples were collected randomly on eight separate locations with a total of 800 g and pooled before the analysis. Then, the soil density was derived from the total organic carbon available, as showed in Equation 5 where ρ is the soil density ($\text{Mg} \cdot \text{m}^{-3}$) and $\text{TOC}\%$ is the concentration in the percentage of organic C (Hollis and Woods 1989), in order to compute the carbon stock (SOC), applied from Papais et al. (2014) and reported in Equation 6.

$$\rho = -0.00745 \cdot \text{TOC}\% + 0.593 \quad (5)$$

$$\text{SOC}_{\text{soil}} = \sum_{n=1}^k [\text{TOC}\% \cdot \rho \cdot T \cdot (1 - \delta) \cdot 10] \quad (6)$$

Where: SOC is expressed in $\text{Mg} \cdot \text{ha}^{-1}$; T is the height of the soil horizon; δ is the coarse fraction in the soil horizon expressed as % of material greater than 2 mm. For this study, SOC was calculated for a scenario on soil of a potential depth of 10 cm.

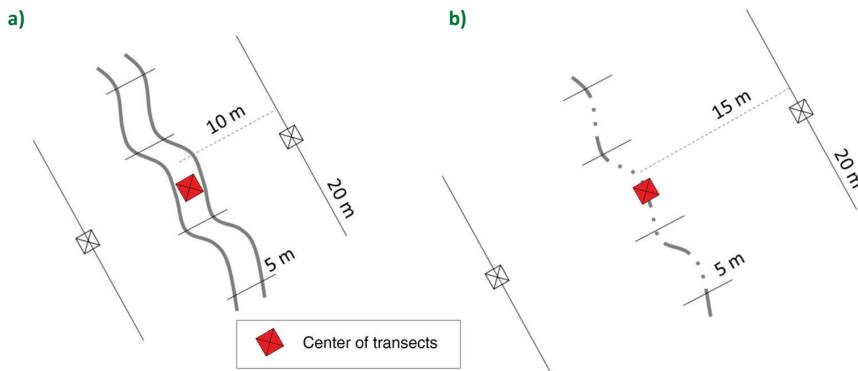


Figure 4. Transect organization and localization referred to (a) harvester-forwarder sites (HF) and (b) cable yarder sites (CY).

Table 2. Time lag class distribution, with diameter (D) thresholds, and category for forest residues (FWD - fine woody debris, CWD - coarse woody debris) (Brown 1974, Rizzolo 2016).

Class	D min (mm)	D max (mm)	Category
A	0	6	FWD
B	6	25	
C	25	76	
D	76	203	CWD
E	> 203		

For the chemical analysis of the residues, a sample for each diameter class was randomly collected on each site from the extraction lines and the areas on the side. After that, the samples from each forest and harvesting systems were regrouped, mixed and grinded, obtaining four samples, one for each diameter class. For CWD, the D and E class have been combined due to the similarity of the material. Specifically for this last sample, a quantification of the carbon stock was conducted since a lower decay rate characterizes it, therefore with potentially a higher carbon input for the soil. The adopted method was the same as by Petrillo et al. (2016), displayed in Equation 7.

$$SOC_{CWD} = \sum_i \frac{V_i \cdot TOC\%_i \cdot \rho_w}{100} \tag{7}$$

Where i refers to each decay class considered; V is the volume per hectare; and pw is the wood density (g·cm⁻³).

RESULTS

Residues’ Quantity According to Harvesting Systems

The estimated mass per hectare for HF sites is reported in Table 3. For the time period considered it emerges that, despite class A with similar values, the tendency for the contents in the other classes is to decrease with time. The higher estimated average value remains for the residues in

class C. For the coarser classes, the estimated mass showed higher values increasing throughout the years, with increasing standard deviation, and therefore variability as well. A similar trend emerges by looking at the median values (Table 4).

The mass per hectare estimation for CY sites is reported in Table 5. Compared to HF, for the cable yarder sites the emerging trend is different: for each year, the highest quantity belongs to class D, except for 2020 that is class C. Considering a broader spectrum, CWD values for CY increase through the years in the considered period. The coarser material (class E) registered the highest values of standard deviation, indicating larger variability among the sampled material. A similar trend is highlighted by the median values reported in Table 6.

The residues’ spatial distribution is shown in Figure 5a and 5b, for HF sites and CY sites, respectively. For HF sites, greater variability within residues can be observed in classes with bigger dimensions. For each year and class of FWD (A, B and C), more material is found in the central transects rather than the lateral ones. The same can be said for class D, but the tendency overturns for the coarse material of class E, where there is more material on the lateral transects. For the CY sites there is much more variability in terms of estimators’ values and material distribution, which generally increases through the years both for central and lateral transects.

Table 3. Average mass value (Mg·ha⁻¹) for residues (FWD - fine woody debris, CWD - coarse woody debris) in harvester-forwarder (HF) sites divided for each class. Standard deviation is also reported in brackets.

Average mass value	FWD			CWD		Sum (Mg·ha ⁻¹)
	A (Mg·ha ⁻¹)	B (Mg·ha ⁻¹)	C (Mg·ha ⁻¹)	D (Mg·ha ⁻¹)	E (Mg·ha ⁻¹)	
HF 2019	1.79 (1.25)	9.77 (4.06)	19.33 (10.80)	13.01 (9.86)	9.59 (17.84)	53.49
HF 2020	2.49 (0.94)	10.04 (4.32)	14.74 (14.97)	8.61 (10.48)	14.24 (20.20)	50.12
HF 2021	1.93 (0.98)	7.68 (3.56)	10.64 (3.26)	9.66 (6.27)	22.43 (28.99)	52.34

Table 4. Median mass values (Mg·ha⁻¹) for residues (FWD - fine woody debris, CWD - coarse woody debris) in harvester-forwarder (HF) sites divided for each class.

Median value	FWD			CWD	
	A (Mg·ha ⁻¹)	B (Mg·ha ⁻¹)	C (Mg·ha ⁻¹)	D (Mg·ha ⁻¹)	E (Mg·ha ⁻¹)
HF 2019	1.56	10.04	19.18	14.02	0.00
HF 2020	2.44	11.28	9.48	9.07	6.28
HF 2021	2.05	6.92	11.30	9.17	13.73

Table 5. Average mass value ($\text{Mg}\cdot\text{ha}^{-1}$) for residues (FWD - fine woody debris, CWD - coarse woody debris) in cable yarder (CY) sites divided for each class. Standard deviation is also reported in brackets.

Average mass value	FWD			CWD		Sum ($\text{Mg}\cdot\text{ha}^{-1}$)
	A ($\text{Mg}\cdot\text{ha}^{-1}$)	B ($\text{Mg}\cdot\text{ha}^{-1}$)	C ($\text{Mg}\cdot\text{ha}^{-1}$)	D ($\text{Mg}\cdot\text{ha}^{-1}$)	E ($\text{Mg}\cdot\text{ha}^{-1}$)	
CY 2019	2.74 (1.21)	7.19 (2.60)	13.45 (10.45)	15.16 (7.57)	4.60 (9.57)	43.14
CY 2020	2.97 (1.38)	10.72 (5.18)	14.01 (5.39)	11.72 (9.34)	12.51 (16.14)	51.93
CY 2021	6.69 (3.55)	15.73 (7.07)	16.37 (8.43)	21.68 (13.99)	9.61 (14.04)	70.08

Table 6. Median mass value ($\text{Mg}\cdot\text{ha}^{-1}$) for residues (FWD - fine woody debris, CWD - coarse woody debris) in cable yarder (CY) sites divided for each class.

Median value	FWD			CWD	
	A ($\text{Mg}\cdot\text{ha}^{-1}$)	B ($\text{Mg}\cdot\text{ha}^{-1}$)	C ($\text{Mg}\cdot\text{ha}^{-1}$)	D ($\text{Mg}\cdot\text{ha}^{-1}$)	E ($\text{Mg}\cdot\text{ha}^{-1}$)
CY 2019	1.94	7.06	8.09	15.95	0.00
CY 2020	2.79	8.73	14.00	9.29	0.00
CY 2021	6.04	14.85	14.05	20.01	8.98

Differences Between Residue Mass Estimators

Table 7 summarises the *p*-value computed with the test divided by year of operation and classes. Overall, for the quantities of the first and the second year (2019 and 2020, respectively), there are no significant differences that can be appreciated among the two harvesting systems. The values for the third year (2021), on the other hand, display a different outcome: the *p*-value computed for 3 out of 5 estimators resulted with the highest level of significance ($p < 0.01$), highlighting the differences between the harvesting systems.

Chemical Analysis of Soil and Residues

The results of the chemical analysis are presented in Table 8 for both soil and residues. As for the soil, San Martino presented a higher moisture content compared to the other two. For both total nitrogen and total carbon, there is a decreasing trend with the decreasing moisture content of the sample. This is also reflected in the organic fraction and with less degree for the inorganic one.

The differences in the soil chemical composition are shown in Table 9. In particular, related to the Cadino forest, with the exception of P, all the other elements concentration are lower compared to other forests. Moreover, although the differences between Paneveggio and San Martino are not evident, and despite the closer geographic location, there is a higher concentration of Ca, Mg and Mn in the first one, and higher concentration of N, C, Fe, K and P in the second one. For the carbon stock, with a hypothetical organic horizon of 10 cm, the forests of Cadino showed the lower value ($45 \text{ Mg}\cdot\text{ha}^{-1}$) and San Martino the highest ($85 \text{ Mg}\cdot\text{ha}^{-1}$).

The same analysis was repeated on the residues and reported in Table 10. The general tendency for all the elements is to maintain a higher concentration on the finer residues, decreasing with the increasing size of material.

A potential SOC was computed for the CWD classes as aggregated information, as a possible contribution of organic carbon that can be absorbed in the soil once the material is decomposed (Table 11). The potential SOC for the 2019 and 2020 sites have similar values, with a higher value for both 2021 sites.

Table 7. A summary of the *p*-values calculated with the two-tailed Mann-Whitney U-test between cable yarder (CY) and harvester-forwarder (HF) sites for each year of operation. The time in years from the Vaia storm is reported in brackets.

	FWD			CWD	
	A	B	C	D	E
2019 (1)	0.0260	0.9213	0.9533	0.2131	0.6038
2020 (2)	0.2981	0.4648	0.1657	0.1420	0.6268
2021 (3)	0.0013*	0.0040*	0.0560	0.0067*	0.9370

Notes: *' highlights the significant level considered. FWD -fine woody debris; CWD – coarse woody debris.

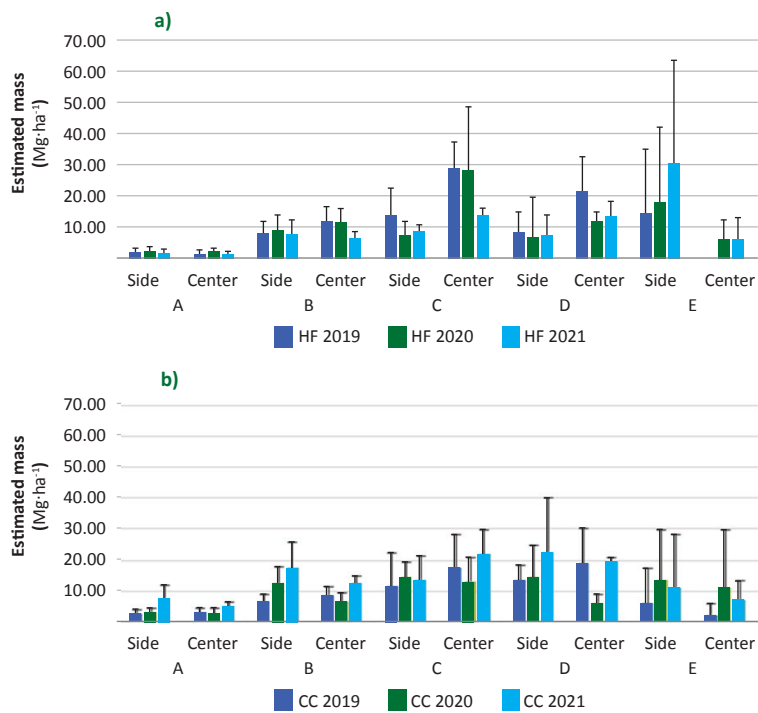


Figure 5. A comparison between average mass estimators for **a)** harvester-forwarder (HF) sites and **b)** cable yarder (CY) sites. The figure shows lateral areas (side) and extraction line (central) with the standard deviation reported in values as bars but represented only on the positive side.

Table 8. Dry matter (d.m.), nitrogen and carbon content resulted from the chemical analysis.

Analysed sample	Denomination	Total dry matter (%)	Total nitrogen (% d.m.)	Total carbon (% d.m.)	Total organic carbon (% d.m.)	Total inorganic carbon (% d.m.)
Soil	San Martino di C.	52.72	0.94	18.70	18.59	0.11
	Paneveggio	64.46	0.74	12.75	12.63	0.11
	Cadino	60.93	0.52	8.65	8.58	0.07
Residues	A	87.41	1.09	56.18	55.98	0.21
	B	84.44	0.40	53.46	53.31	0.14
	C	87.22	0.39	53.24	53.08	0.16
	D/E	88.06	0.43	51.98	51.85	0.12

Table 9. A summary of the chemical analysis for the three forests soils.

Element	Unit	San Martino di C.	Paneveggio	Cadino
N	g·kg ⁻¹ d.m.	9.41	7.35	5.20
C organic	g·kg ⁻¹ d.m.	185.86	126.34	85.80
Ca	mg·kg ⁻¹ d.m.	2,054.58	3,577.62	1,299.39
Fe	mg·kg ⁻¹ d.m.	22,152.22	20,637.05	15,272.03
K	mg·kg ⁻¹ d.m.	4,794.01	4,677.20	3,349.72
Mg	mg·kg ⁻¹ d.m.	3,821.68	4,294.74	1,991.27
Mn	mg·kg ⁻¹ d.m.	267.18	455.77	201.66
P	mg·kg ⁻¹ d.m.	576.63	395.21	384.75
Carbon stock	Mg·ha ⁻¹	85	63	45

Table 10. Average nutrient values for each residue diameter class across the three forests.

Element	Unit	A	B	C	D/E
N	g·kg ⁻¹ d.m.	10.86	4.05	3.92	4.30
C organic	g·kg ⁻¹ d.m.	559.76	533.15	530.75	518.51
Ca	mg·kg ⁻¹ d.m.	6,566.98	3,197.47	3,525.09	1,227.86
Fe	mg·kg ⁻¹ d.m.	300.73	81.71	24.21	46.23
K	mg·kg ⁻¹ d.m.	1,306.38	332.58	512.07	365.57
Mg	mg·kg ⁻¹ d.m.	616.37	325.67	207.80	123.72
Mn	mg·kg ⁻¹ d.m.	310.38	163.82	128.02	64.28
P	mg·kg ⁻¹ d.m.	666.34	108.56	84.60	50.9

Table 11. Potential SOC stocked in coarse woody debris (CWD) quantity for each salvage logging site, cable yarder (CY) and harvester-forwarder (HF).

Salvage logging site	CWD (D/E) (Mg·ha ⁻¹)	SOC (Mg·ha ⁻¹)
HF 2019	23.80	11
HF 2020	22.85	10
HF 2021	32.09	15
CY 2019	21.08	10
CY 2020	24.23	11
CY 2021	31.29	14

DISCUSSION

Residues' Quantity According to Harvesting Systems

Overall, throughout the years, the amount of material for HF sites is similar, whereas for CY sites it is increasing. There is an increasing trend for the coarser material (class E) for both harvesting systems. The total quantity of residues is greater in the first year after the storm (2019) for HF sites than for CY. This information can therefore be considered similar to that from conventional clear-cuts (non-salvage logging conditions), where the CTL system releases more residues than FT (Hytönen and Moilanen 2014). In the following two years, the residue amounts computed for CY sites are greater than for HF, showing some marked differences. However, from the statistical point of view, no significant differences can be found between the first two years regarding the adopted systems. Moreover, significant differences emerged for the salvage logging operations occurring three years after the windthrow event. Based on the hypothesis stated, those difference should be reconducted to the choice of harvesting system and machine configuration. However, considering the results and site conditions, the influence of other factors, like the change in moisture content of the wood, should not be excluded, which may result in possible degradation (Petrillo et al. 2016) and increasing susceptibility to breakage, or the selection process of retrieving the material performed by the operators.

The fully mechanized machine configuration has a direct influence on the first two classes (A and B), which can be attributed to the transit of the forest machines (harvester and forwarder) when compared to the cable yarder full tree extraction. The passage over the logging residues causes mixing of the soil that eventually incorporates finer dead wood

material, with subsequent compaction, preventing it from being correctly counted in the survey. Moreover, in HF sites, the material is processed in front of the vehicle, concentrating the logging residues on the trail, especially branches and tops producing a brush mat to reduce soil deformation and risk of erosion (Borchert et al. 2012). In contrast, the coarser material like stumps or bigger logs are deposited on the side of the same trails. This trend is particularly visible for classes B, C and D in Figure 5a. This distribution is due to the machine processes, as this type of material is usually found in higher concentrations in the working area of the machine, where the central transect is located. In the case of a harvester, residual material is in the front of the machine, whereas for stumps and coarser material (class D/E material) are usually left outside the working area.

For CY sites, on the other hand, the difference between side trails and the centre is not yet so marked (Figure 5b). The absence of a clear trend in terms of residue concentrations between lateral and central transects can be justified by the use of the FT system, where the entire tree is extracted from the forest and the majority of residues are then found at the roadside. However, the increase in the third year (2021) can be attributed to dragging in the phases of concentration and the removal of the material to cause the loss of branches and twigs. This is particularly visible in Tables 5 and 6 for finer material (A and B classes). Here the emerging differences can be reconducted to the change in the material moisture content, degradation and decomposition, especially the logs already in contact with the ground. The desiccation and subsequent loss of elasticity of the branches can lead to increasing biomass on the forest floor. These observations, without the possibility of adequately mapping the spatial distribution of residues, suffer from uncertainties, and a remote sensing approach could improve this information (Udali et al. 2022).

Growing stock, tree height and spatial distribution, together with the high gap fraction, might have some influence as well: the bigger quantity of CWD, for example, can be linked with the tree height present since usually the tallest trees also possess high diameter. This can be perceived, to some extent, for both HF and CY.

Other differences, for example for CWD, are presumed to be caused by the selection process in the sorting and extraction operations, and are thus related to the system-configuration choice. These operations are performed in the salvage logging site by the forwarder operators (CTL) – therefore leaving more material on the ground – and by the

ground crew (FT) – when extracting the whole tree or part of it from the forest to roadside. The effects can be observed for the considered category of residues in Table 3 and Table 5 with the highest values in third year (2021) after the storm.

Chemical Analysis of Soil and Residues

In particular, the values computed for SOC align with those presented for Alpine forests by Gachhadar et al. (2022). Moreover, they also reported an average value of $94.95 \text{ Mg}\cdot\text{ha}^{-1}$, which resulted in higher values than those obtained with almost $85 \text{ Mg}\cdot\text{ha}^{-1}$ from the forest in San Martino. Overall, this is valuable information considering that the soil depth in the Alpine forests is not remarkably high and the average altitude of the study sites (1,550–1,650 m a.s.l.). Although not easily detectable, in HF sites the mixing of soil horizons with residues might reduce nutrient losses and leaching (especially N), but also can lead to an increment in erosive processes (Merino et al. 1998). This also finds correspondence in the study by Huber et al. (2017), where FT cable yarding harvesting increased nitrogen removal compared to CTL-harvesting systems.

Considering the CWD material, the values observed are in line with the ones reported in previous studies in the European context, ranging between 50 and $120 \text{ m}^3\cdot\text{ha}^{-1}$ (Gutowski et al. 2005). The estimators are also comparable with the results obtained by Petrillo et al. (2016) in similar areas in the Province of Trento. First of all, the chemical analysis revealed higher concentrations for all the elements considered for the sites, including carbon. This information could be relevant considering the salvage logging operations running in the areas prior the survey versus the undisturbed conditions of the areas surveyed. Moreover, the SOC for CWD material computed by Petrillo et al. (2016) ranged between 3 and $17 \text{ Mg}\cdot\text{ha}^{-1}$, with an average value of south-facing sites of $8.25 \text{ Mg}\cdot\text{ha}^{-1}$, lower than the one obtained for the south-facing sites considered in this study ($11 \text{ Mg}\cdot\text{ha}^{-1}$).

Practical Outcomes

The effects of salvage logging operations on logging residues were assessed over a short time span (3 years) after the storm event. Combining the observations reported above and others from the literature, some practical considerations can be made.

i. Conditions. The scenario in which these operations have been conducted is of non-conventional conditions since high severity disturbance affected the stands. Salvage logging has taken place with the main intent to reduce the economic loss, but also to reduce the risk for the subsequent event, such as fires or bark beetle outbreak (Leverkus et al. 2021). In these areas traditional logging operations are normally performed using single tree selection or cut-block, and with less impacting systems (Lindenmayer and Noss 2006), preferring motor-manual operations and extraction using either tractor and winch or cable yarders.

ii. Harvesting systems (1). In more standard conditions, CTL systems should leave more residues on site than FT (Hytönen and Moilanen 2014), and this can be assessed by looking at the first year's residue quantities (2019) presented in this study. However, when it comes to salvage logging, this relation might not al-

ways be proved true. Both CTL and FT systems can be adopted through different degrees of mechanization, therefore having a variable impact.

iii. Harvesting system (2). The general recommendation is to adopt highly mechanized systems using CTL systems (Sanginés de Cárcer et al. 2021). This to ensure primarily the operator safety and to quickly recover the damaged timber. In this case, ground-based systems, such as the harvester-forwarder configuration, might be considered the optimum in the short term to maintain higher productivity and to reduce an economic loss (Udali et al. 2021). Also, ground-based system can mix up the residues within the soil horizons, and this can reduce nutrient loss. However, their passage and deep ruts can enhance nutrient leaching (especially N) and erosive processes (Merino et al. 1998).

iv. Timing of operations. The need to perform salvage logging operations immediately after the disturbance event is mainly needed to ensure the safety of infrastructure and to recover the economic value of timber. However, the planning of operations should also consider future impacts on site fertility: the extensive removal of residues, for example, affects tree growth and wood density (Roy et al. 2022). Both harvesting systems can be considered appropriate options to avoid nutrients losses, with the possibility to implement FT into a partial extraction of the full tree with the release of the treetop and the branches, leaving behind most of the needles and, potentially, the majority of nutrients. Furthermore, a lighter carriage can develop less tension on the mainline and reduce the overall forces on the system, making it safer for the operators. Adopting FT systems from the second year (i.e., after trees have lost their needles) can be considered a solution, as shown in this study, along with leaving behind a certain volume of finer residues (Nilsson et al. 2018).

v. Effects of residues. The release of residues should be weighted on a different number of elements, all site-specific. In the Alpine environment many ecosystem services granted by the forest do not have a direct market value (Häyhä et al. 2015). However, they can also be provided by the presence of residues. For example, logging residues can increase the presence of favourable microsites for regeneration, enhancing site characteristics by lowering high diurnal temperature and maintaining higher soil moisture (Marangon et al. 2022). Their presence, especially CWD, reduces the risk for gravitational hazards playing an active role against rockfalls (Costa et al. 2021). Moreover, the retention of residues also helps reduce the risk of erosion, especially on skid trails (Mazri et al. 2020).

CONCLUSIONS

The study aimed to verify the presence of differences in terms of logging residue type and quantity according to the harvesting system adopted in the case of a salvage logging operations. A second focus was put on assessing the effects on

nutrients and possible impacts on carbon stocks for both soil and residues. Moreover, the effect of the timing of operations was also considered in relation to residue quantities. Overall, significant differences have emerged in terms of residues left on site due to the harvesting systems (cut-to-length versus full-tree systems) and the configuration adopted (ground-based versus aerial-based) in later years with respect to the storm event. However, the sole system-configuration combination was not able to explain all the variability between years and sites in the approach considered. Based on this study's outcomes, further investigations should address the residues' spatial distribution after logging operations at greater resolution considering the entire area of operations and the possible effects on nutrients and carbon distribution in the salvaged logging areas.

Author Contributions

AU, LG, EL, SG conceived and designed the research; LG carried out the field measurements; LG and AU processed the data and performed the statistical analysis; EL and RC revised and contributed to the discussion of results; AU, LG, SG wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Occurrence of Bees and Bumblebees in Bark Beetle Slit Traps from Spruce and Fir Woodlands of Central Dinaric Alps

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ABSTRACT

The paper analysed bees by-catch collected in 259 bark beetle slit traps, from eleven localities in Bosnia and Herzegovina. Sampling was carried out in spruce and fir forests in 2020 and 2021. As a by-catch from bark beetle slit traps 84 bee individuals from four families and 13 genera were collected. In the bark beetle slit traps sample, out of 29 bee taxa, 22 species were identified at the species level and eight specimens were left at the genus/subgenus level. The most dominant genera were *Megachile* with 34 specimens and *Osmia* represented by 20 specimens in the total sample. The research identified 14 bee species new to the fauna of Bosnia and Herzegovina. The bee species collected in the bark beetle slit-traps were dominated by nesters in cavities, above the ground-nesting bees.

Keywords: conservation; pollinators; by-catch; forest; management

INTRODUCTION

In Europe, bees represent the main group of pollinators (Drossart and Gérard 2020) and in the European Union pollination has an estimated economic value of €15 billion per year (Nieto et al. 2014). The number of bee species in the wild has declined globally, the decrease occurred in the last 10-15 years, with roughly 25 per cent fewer species recorded (Potts et al. 2010, Zattara and Aisen 2021). The loss of bee diversity has intensified efforts to develop methods of standardized sampling and the assessment of bee diversity. The three most commonly used trapping methods for collecting bees for biodiversity studies are bowl traps, vane traps, and Malaise traps. Methods of passive bee sampling with traps and statistical models for the purpose of monitoring are still being developed and attempts are being made to understand the scope of bee diversity that is included through sampling with coloured pan traps (McCravy 2018). Bees also find their way into traps that use both visual and olfactory cues to attract pest insects. Researchers work to improve pest monitoring tools to increase target captures and reduce bee by-catch. The bee by-catch composition analysis can help assess biodiversity, determine population

fluctuations and range expansions, support monitoring efforts, and identify patterns and processes of broader ecological interest (Spears et al. 2021). Forestry implements traps to control bark beetles, ambrosia beetles, wood-boring insects, and wood wasps, thereby minimizing their populations. Additionally, traps in forestry are employed to identify and monitor the presence of pests and invasive insects. Introduced in the late 1970s, pheromone traps were implemented as a replacement for trap trees that had been utilized for over two centuries, serving as a protective measure against the spruce bark beetle (Zahradnik 2015).

Standardised bark beetle slit traps used for the mass trapping of bark beetles can be used with or without an attractant. Bark beetle slit traps are considered to have relatively few wider ecosystem effects on the woodland environment, but this is rarely tested in field conditions. In Bosnia and Herzegovina, monitoring of wild bees by standardized sampling has not been performed and data on the composition of solitary bee and bumblebee fauna from traps are unknown.

The importance of by-catch bees in traps for monitoring of pests has already been confirmed and data were used for biodiversity assessment (Buchholz et al. 2011, Spears and

Ramirez 2015). By-catch from lepidopteran traps and pitfall traps were used to determine the abundance and diversity of bees (Hatten et al. 2013, Hung et al. 2015, Parys et al. 2021).

Considering the importance of solitary bees and population decline trends, data on collected species from non-target catches are a significant source of data on the distribution and diversity of bees, especially if one takes into account the standardized collection method and long-term monitoring of bark beetles in forestry. This study aimed to determine the composition of honey bee, solitary bees and bumblebees that occur in the bark beetle slit traps in the spruce and fir forests of central Bosnia and Herzegovina.

MATERIALS AND METHODS

Control and monitoring of bark beetles are carried out by the University of Sarajevo - Faculty of Forestry, Plant Protection Laboratory. Collected bees were separated from the sample within a bark beetle monitoring program. The by-catch was processed at the Faculty of Science, Department of Biology, where taxonomic analysis of the bees was carried out. Bees from the by-catch were washed, dried and placed on entomological pins. The identification was carried out using stereo zoom microscope with 90X magnification and taxonomic keys (Friese 1895a, 1985b, 1896, 1897, Brohmer et al. 1930, Warncke 1968, Mauss 1994, Amiet et al. 2001, 2004; 2007, 2010, 2014, 2017, Michez et al. 2019, Rasmont et al. 2021). The current systematic and species status follows Kuhlman et al. (2023).

The samples were collected from 259 bark beetle slit traps with attractants; Pheroprax® (ipsdienol, cis-verbenol, 2-methylbut-3-en-2-ol) and Gallowit® (ipsdienol CAS 1443441-4, ipsenol CAS 60894-96-4, DMWK CAS 115-18-4, cis-verbenol CAS 18881-04-4, α-pinene CAS 80-56-8, ethanol CAS 64-17-5). On the sampling sites we use "Theysohn" (producer THEYSOHN Kunststoff GmbH, Germany) type pheromone traps. All traps had the same set of baitpheromones, baited-traps were set approximately 20m from the forest edge, the distance between baited traps was approximately 20 m apart and bark beetle slit traps were placed 1.5 m above the ground.

The sampling was carried out from May to June 2020 and May to September 2021. The pheromone-baited traps were emptied weekly.

Study Sites

Sampling was performed at 11 monitoring sites in central Bosnia and Herzegovina (Figure 1, Table 1).

The monitoring sites are located on Mt. Bjelašnica, Mt. Igman, Mt. Ozren, Mt. Trebević, and Mt. Zvijezda. The traps were used for annual bark beetle control and monitoring. All selected sampling sites were within *Picea abies* (L.) Karst. and *Abies alba* Mill. forests in the Dinaric, Pre-Alpine region (Table 1). Local habitat parameters were not estimated on the field due to a lack of field protocol. Values of climatic parameters for each sampling site were extracted from WorldClim raster for each locality (Fick et al. 2017). The parameters used for obtaining bioclimatic data were extracted from WorldClim raster with a spatial resolution of 1 km²: Annual Mean Temperature - bio1, Mean Temperature of Warmest Quarter - bio10, Mean Temperature of Coldest Quarter - bio11, Annual Precipitation - bio12 (Fick et al. 2017).

Landscape Pattern Analysis

Land cover maps of the study area were generated from Corrine Land Cover maps with 100X100 meters resolution. The classification of the study region was made according to CLC classification. From the CLC map, 11 forest patches were selected. The centre of each CLC patch coincides with the centroid of traps in the investigated localities, calculated using a centroid point layer in QGIS. The forest patch had a diameter of 1600 meters and an area of 2.0 km². Landscape pattern analysis was conducted with QGIS raster to calculate forest cover and landscape heterogeneity using the Shannon diversity index.

The Shannon diversity index considers the number of different types of environments and their proportion in each landscape (Table 2.). If two landscapes are covered by exactly the same types of habitats, that with the highest Shannon-Weaver value will be the one with the highest category evenness (McGarigal et al. 2012).

Data Processing

For each sampling site, we estimated two bee community level variables: richness (number of species), and

Table 1. The position of monitoring sites; coordinates are centroids for bark beetle slit raps used for forest pest control and management

Locality	Lat.	Lon.	Meters
1. Bijambare	44.082868	18.511551	980
2. Gornjebosansko, Gornja Ljubina	43.973329	18.381129	612
3. Trnovo, Hojta-Presjenica	43.674577	18.334640	938
4. Gornjebosansko, Kaljina-Bioštica	44.061094	18.534055	1030
5. Igman	43.752618	18.267648	1240
6. Igmanско, Hadžići Zujevina	43.726973	18.082502	944
7. Skakavac, Vogošća-Bulozi; Vučja Luka	43.939597	18.453573	1409
8. Trebević	43.834989	18.452661	1086
9. Trnovo, Crna Rijeka-Željeznica	43.659614	18.381594	1121
10. Trnovo, Gornja Rakitnica	43.655270	18.286679	1220
11. Gornjebosansko, Gornja Misoča	43.958932	18.308820	757

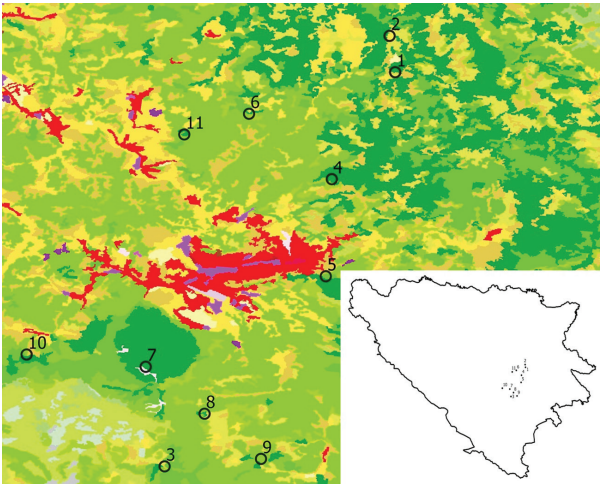


Figure 1. Position of monitoring sites for bark beetle slit traps on Corine Land Cover, circles represent forest patches 2 km² with the associated localities that are numbered, the sampling sites are marked on a blind map of Bosnia and Herzegovina; 1 – Bijambare, 2 - Gornjebosansko, Gornja Ljubina, 3 – Trnovo, Hopta-Presjenica, 4 – Gornjebosansko, Kaljina-Bioštica, 5 – Igman, 6 – Igmansko, Hadžići Zujevina, 7 – Skakavac, Vogošća-Bulozi, Vučja Luka, 8 – Trebević, 9 – Trnovo, Crna Rijeka-Željeznica, 10 – Trnovo, Gornja Rakitnica, 11 – Gornjebosansko, Gornja Misoča.

abundance (total bee amount), from which we calculate the Shannon diversity index. The frequency of bee species in the investigated locality was analysed as an indicator of the diversity and composition of bee communities. Based on the data, the similarity between the samples was compared using Beta Diversity and Pairwise comparison Whittaker. The diversity indexes by sampling locality and the number of bees collected in the traps were used to calculate: Taxa_S, Individuals, Dominance_D, Simpson_1-D, Shannon_H, Margalef, Fisher_alpha, Berger-Parker, chao1. The correlation between altitude, the number of individuals and the number of species was tested using linear correlation.

Ordinary Least Squares Regression was used for the correlation between the number of bees and the number of traps per locality.

RESULTS AND DISCUSSION

The relative abundance of spruce and fir forests according to CLC analysis within an area of 2 km² varies in the range of 0.07-0.88, median=0.31 (Table 2).

The mean values for 11 sampling sites were calculated using data obtained from WorldClim rasters mean±standard deviation (min, max): Average Annual Mean

Table 2. The number of different land cover types, CLC category proportion in each sampling locality and Shannon-H value for each sampling locality.

CLC category	Locality										
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11
Mapped 1.6 km radius landscapes											
142 Sport and leisure facilities	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00
231 Pastures	0.24	0.36	0.00	0.00	0.14	0.00	0.25	0.00	0.00	0.00	0.00
242 Complex cultivation patterns	0.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
243 Annual crops associated with permanent crops	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.21	0.00	0.00
311 Broad-leaved forest	0.00	0.16	0.00	0.17	0.29	0.00	0.51	0.93	0.26	0.53	0.00
312 Coniferous forest	0.27	0.08	0.85	0.31	0.21	0.69	0.24	0.07	0.39	0.47	0.88
313 Mixed forest	0.00	0.40	0.00	0.39	0.082	0.00	0.00	0.00	0.14	0.00	0.00
324 Transitional woodland shrub	0.00	0.00	0.15	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.12
Number of categories	3	4	2	4	5	2	3	2	4	2	2
Shannon-H	1.04	1.24	0.42	1.29	1.52	0.62	1.03	0.25	1.32	0.69	0.37

Temperature °C = 7.32±1.01 (5.65, 9.15), Mean Temperature of Warmest Quarter °C = 15.65±1.15 (13.77, 17.78), Mean Temperature of Coldest Quarter °C = -1.17±0.77 (-2.41, 0.23) and Annual Precipitation mm·m⁻² = 1078,83±30.08 (1035, 1115).

The solitary bees and bumblebees collected in the by-catch sample from the bark beetle slit-traps were represented by 84 bees. In 2020, 19 bees were collected from 18 bark beetle slit traps and in 2021, 65 bees were collected from 39 slit traps.

The minimum number of bees in the bark beetle slit traps was collected in May 2021, the bees were most numerous in July 2020 - 10 bees and in July 2021 - 23 bees. The number of bees in traps decreases in August and September (Table 3). The maximum number of individuals from the genus *Osmia* was collected in the traps in July and June, while the genus *Megachile* was most dominant in July and August (Table 3.).

The sampled bees collected in bark beetle slit traps belong to 13 genera (Table 3). The prevailing bee genera was *Megachile* represented by 34 individuals, *Osmia* represented by 20 bees and *Apis* - 10 individuals (Table 3). The genus *Megachile*, *Osmia* and *Apis* in the sample comprise 76% of the total sample. *Apis mellifera* was represented in 73% of the sampling localities. In the total sample, 49 bees were females and 35 were males. The ratio of males to females in the genus *Megachile* was in favor of males, while in the genus *Osmia* females were dominant (Table 4).

Three localities stand out regarding the number of collected specimens: locality 5 Igman (16), locality 7 Skakavac, Vogošća-Bulozi, Vučja Luka (15) and locality 10 Trnovo, Gornja Rakitnica (17). The aforementioned localities account for 57.14% of the collected bees in bark beetle slit traps (Table 5). The localities with the largest number of collected bees are the ones with the highest species diversity, which is locality 7 Skakavac, Vogošća-Bulozi, Vučja Luka (Table 4).

Genus *Osmia* was represented by eight species and two bees which were identified at the genus level. The genus

Megachile was represented by seven species and one bee was identified at the genus level. The lowest number of identified individuals at the species level from the slit traps was in the genus *Bombus*. Due to the damage of the individuals in the bark beetle slit-traps, only one bumblebee was identified at the species level (Table 5.).

Diversity indices show the localities which are richest in diversity are: 5 Igman, 7 Skakavac-Vogošća-Bulozi-Vučja Luka, and 10 Trnovo-Gornja Rakitnica (Table 6.).

The relationship between altitude, the number of individuals and the number of bee species has a positive correlation $r=0.61$; $p=0.037$. Ordinary Least Squares Regression showed a statistically significant correlation between the number of individuals and the number of traps per locality: $t=2.77$, $p=0.02$, $r=0.64$, $r^2=0.41$. The number of collected individuals and the number of traps per locality is different; the largest number of traps is in the Igman locality with 93 traps, and in the Trnovo, Crna Rijeka-Željeznica locality two traps were present (Table 7).

From 259 bark beetle slit traps, we collected 84 bees belonging to 26 species. The number of ground-nesting solitary bees and bumblebee species was seven. Overground cavity-nesting bees and dead wood nesters were represented by 18 species and *Apis mellifera* was a eusocial cavity-nesting bee. The number of dead wood nesting bees and over-ground cavity nesters (78) was greater than the number of ground-nesting bees in the by-catch sample. We also collected parasitic bees: *Coelioxys conica*, *Sphecodes majalis* and *Stelis punctulatissima*. Megachilidae were the most abundant taxonomic group represented by 17 bees identified to the species level. The relationship between Megachilidae and bark beetle slit traps placed over the ground is based on the biology of a group that nests in cavities above ground, most often in pre-existing abandoned tunnels of saproxylic insects. The data regarding collected bees is significant from the aspect of understanding the diversity of local bee fauna. The checklist for bee fauna for Bosnia and Herzegovina lists 125

Table 3. Variation in the number of collected bees in bark beetle slit traps during the sampling period July-August 2020 and May-September 2021, dates are in year/month format.

Family	Genus	2020/07	2020/08	2021/05	2021/06	2021/07	2021/08	2021/09	Total
Andrenidae	<i>Andrena</i>	0	0	0	0	0	2	0	2
Apidae	<i>Apis</i>	0	2	0	4	3	1	0	10
Apidae	<i>Bombus</i>	3	3	0	0	1	0	0	7
Apidae	<i>Eucera</i>	0	0	0	0	1	0	0	1
Halictidae	<i>Dufourea</i>	0	0	0	1	0	0	0	1
Halictidae	<i>Halictus</i>	0	0	0	0	0	0	1	1
Halictidae	<i>Sphecodes</i>	0	0	0	1	0	0	0	1
Megachilidae	<i>Anthidium</i>	1	1	1	0	0	1	0	4
Megachilidae	<i>Coelioxys</i>	1	0	0	0	0	0	0	1
Megachilidae	<i>Hoplosmia</i>	0	0	0	0	1	0	0	1
Megachilidae	<i>Megachile</i>	3	2	0	3	10	10	6	34
Megachilidae	<i>Osmia</i>	1	1	1	7	7	1	2	20
Megachilidae	<i>Stelis</i>	1	0	0	0	0	0	0	1
Total		10	9	2	16	23	13	9	

Table 4. The number of collected bee specimens in bark beetle slit-traps by genus and gender.

Genus	Number of specimens	Males	Females
1. <i>Andrena</i>	2		2
2. <i>Apis</i>	10	2	8
3. <i>Bombus</i>	7		7
4. <i>Eucera</i>	1		1
5. <i>Dufourea</i>	1	1	
6. <i>Halictus</i>	1	1	
7. <i>Sphecodes</i>	1	1	
8. <i>Anthidium</i>	4	1	3
9. <i>Coelioxys</i>	1	1	
10. <i>Hoplosmia</i>	1	1	
11. <i>Megachile</i>	34	20	14
12. <i>Osmia</i>	20	6	14
13. <i>Stelis</i>	1	1	
Number of specimens	84	35	49

species (Apfelbeck 1896). Comparison with a bee checklist of Serbia (Mudri-Stojnić et al. 2021) with 706 species, indicates a significant difference in species diversity. Of the total 22 identified bee species in the study, up to date, 14 species have not been recorded for Bosnia and Herzegovina according to Apfelbeck checklist: *Andrena tscheki*, *Bombus sylvestris*, *Coelioxys conica*, *Megachile centuncularis*, *M. genalis*, *M. pildens*, *M. pilicrus*, *Osmia bicornis*, *O. claviventris*, *O. latreillei*, *O. leaiana*, *O. mustelina*, *Sphecodes majalis*, *Stelis punctulatissima*.

The relationship between altitude, the number of individuals and the number of bee species has a positive correlation $r=0.61$; $p=0.037$. Multivariate linear regression analysis correlated habitat heterogeneity with the number of bee species, the number of collected bees and Shannon – H diversity index for each investigated locality (Table 8). Linear regression analysis indicated a negative correlation between habitat heterogeneity and the number of bee species; habitat heterogeneity and the number of bees. The positive correlation was found between habitat heterogeneity and Shannon – H for bee diversity, and the correlations had no statistical significance.

The spruce and fir forests dying due to bark beetle infestation changes the forest structure and has a positive effect on the diversity of pollinators. Bark beetle infestation leads to the death of trees and loss of cover in the tree floor, a greater amount of light can stimulate the growth of herbaceous plants. Bees are positively associated with disturbed forest habitats and forest low tree cover with high floral richness, while abundant dead wood creates suitable conditions for bees (Moretti et al. 2009, Williams et al. 2010, Spears and Ramirez 2015). In addition to the aforementioned loss of leaf mass, measures to control bark beetles are reduced to bare cutting and cleaning of infested areas, which opens up forest habitats. The bee species richness in the forests increases with flower richness

and clear-cut size (Taki et al. 2007, Watson et al. 2011, Schüepp et al. 2011). The landscapes with more forests and environmental heterogeneity can provide more resources for bees through resource complementation processes, maintaining their diversity in the landscape. The presence of forest patches close to open areas is of utmost importance for the conservation of bees and pollination services (Rubene et al. 2015). Data related to the wild bees by-catch composition in forest pest management traps, such as bark beetle slit traps in Bosnia and Herzegovina, have not been studied. The absence of long-term bee monitoring can most likely be compensated by the analysis of the composition of the bee fauna in the traps for e.g., bark beetle, as by-catch. By-catches, also, may cause a variety of adverse consequences on populations, food webs and conservation efforts (Revill et al. 2005).

Integrated Pest and Pollinator Management has been proposed as a new framework to further improve the compatibility of pest management practices with pollinator conservation strategies (Biddinger and Rajotte 2015). Considering that integrated pest management and the inclusion of beneficial insects leads to a higher number of insect groups requiring taxonomic identification, the need for collaboration with additional taxonomists for the identification of materials collected from traps becomes crucial. Due to the intricate nature of the task, the challenges highlight the importance of teamwork and synchronized efforts among multiple teams, as well as the regular exchange of materials with expert taxonomists (Spears et al. 2021).

Non-targeted catch has been used to facilitate taxonomic research and describe new species, identify novel invasive alien species, enhance stakeholder knowledge and conduct surveys of non-target insects. The significant presence of *Megachile* and *Osmia* solitary bee species in the sample indicates the need for additional investigation

Table 5. The number of collected bees in traps by locality and nest preference: ground nester (GN), cavity nester over ground (CN), preexisting holes wood nester, or over ground wood nester (WN).

	Bijambare	Gornjebosansko, Gornja Ljubina	Trnovo, Hojta- Presjenica	Gornjebosansko, Kalljina-Bioštica	Igman	Igmansko, Hadžići Zujevina	Skakavac, Vogošća- Bulozi; Vučja Luka	Trebević	Trnovo, Črna Rijeka-Željeznica	Trnovo, Gornja Rakitnica	Gornjebosansko, Gornja Misoča	Number of specimens
1. <i>Andrena</i> sp. (GN)	0	1	0	0	0	0	0	0	0	0	0	1
2. <i>Andrena tscheki</i> (GN)	0	1	0	0	0	0	0	0	0	0	0	1
3. <i>Anthidium manicatum</i> (CN)	0	0	1	0	2	0	1	0	0	0	0	4
4. <i>Apis mellifera</i> (CN)	2	1	0	2	1	0	1	1	0	1	1	10
5. <i>Bombus</i> sp. (GN)	0	0	0	0	4	1	1	0	0	0	0	6
6. <i>Bombus sylvestris</i> (GN)	1	0	0	0	0	0	0	0	0	0	0	1
7. <i>Dufourea</i> sp. (GN)	0	0	0	0	0	1	0	0	0	0	0	1
8. <i>Eucera longicornis</i> (GN)	0	0	0	0	0	0	1	0	0	0	0	1
9. <i>Halictus</i> sp. (GN)	0	0	0	0	0	0	0	0	0	1	0	1
10. <i>Coelioxys conica</i> (WN)	0	0	0	0	1	1	0	1	0	0	0	3
11. <i>Megachile centuncularis</i> (WN)	0	0	1	0	1	0	1	1	0	2	0	6
12. <i>Megachile genalis</i> (WN)	0	0	0	0	1	0	0	1	0	2	0	4
13. <i>Megachile ligniseica</i> (WN)	0	0	0	0	0	0	1	0	0	0	0	1
14. <i>Megachile pildens</i> (WN)	0	0	1	0	0	0	0	1	0	0	0	2
15. <i>Megachile pilicrus</i> (WN)	0	0	0	0	1	0	1	0	0	0	0	2
16. <i>Megachile</i> sp.	1	0	2	0	0	0	3	0	0	2	1	9
17. <i>Megachile willughbiella</i> (WN)	2	0	0	0	0	0	1	1	0	4	0	8
18. <i>Osmia caerulea</i> (WN)	0	0	0	0	0	0	0	0	0	0	1	1
19. <i>Osmia aurulenta</i> (WN)	0	0	0	1	0	0	0	0	0	0	0	1
20. <i>Osmia bicornis</i> (WN)	0	0	0	0	1	0	1	2	0	0	0	4
21. <i>Osmia claviventris</i> (WN)	1	0	0	0	0	0	0	0	1	0	0	2
22. <i>Osmia cornuta</i> (WN)	0	0	0	0	1	0	2	0	0	0	0	3
23. <i>Osmia latreillei</i> (WN)	0	0	0	0	0	0	0	0	0	1	0	1
24. <i>Osmia leaiana</i> (WN)	0	0	0	0	0	0	0	0	0	1	0	1
25. <i>Osmia mustelina</i> (CN)	0	0	0	0	0	0	1	0	0	0	0	1
26. <i>Osmia</i> sp.	0	0	0	0	3	0	0	0	0	3	0	6
27. <i>Hoplosmia</i> sp.	0	0	0	0	0	0	0	0	0	0	1	1
28. <i>Sphecodes majalis</i> (GN)	1	0	0	0	0	0	0	0	0	0	0	1
29. <i>Stelis punctulatissima</i> (WN)	0	0	0	0	0	1	0	0	0	0	0	1
Number of bees	8	3	5	3	16	4	15	8	1	17	4	84

into the effectiveness of bark beetle slit traps for monitoring these genera in the Dinaric Alps.

Further research is needed to verify the species diversity of the genera *Megachile* and *Osmia* in pest monitoring traps compared to traps used in bee biodiversity research, such as pan traps. Establishing a correlation in the species diversity of the aforementioned genera enables the introduction of an integrative approach to the control of pests and bee monitoring using bark beetle slit traps. Collecting data from forest pest management traps is the first step towards better knowledge of the distribution of solitary bees and bumblebees. Taking into account the standard sizes of bark beetle slit traps and a larger number of traps, it is most likely possible to monitor bees and determine the correlation between the forest habitat heterogeneity and community and the diversity of bees.

CONCLUSIONS

Solitary bees (79.8%), bumblebees (8.4%) and honey bees (11.8%) were detected as by catches in bark beetle slit traps used for the control and monitoring of bark beetles. The sample of bees from traps is dominated by species nesting in existing tree cavities: *Osmia* 8/22 (36%) and *Megachile* 6/22 (27%). A positive correlation was established between the number of collected bees and the number of traps per locality. Slit traps for the control of bark beetles are important as a source of information about the bee fauna when there is no long-term research on bee fauna of Bosnia and Herzegovina.

The presence of 14 new species of bees was determined which were not previously recorded for the fauna of Bosnia and Herzegovina. The data on the connection between habitat structure and the number of bee species are contrived and

Table 6. Overview of the diversity indices by locality based on the number of bees collected in the traps.

	Bijambare	Gornjebosansko, Gornja Ljubina	Trnovo, Hojta- Presjenica	Gornjebosansko, Kaljina-Bioštica	Igman	Igmansko, Hadžići Zujevina	Skakavac, Vogošća-Bulozi; Vučja Luka	Trebević	Trnovo Crna Rijeka-Željeznica	Trnovo, Gornja Rakitnica	Gornjebosansko, Gornja Misoča
Taxa_S	6	3	4	2	10	4	12	7	1	9	4
Individuals	8	3	5	3	16	4	15	8	1	17	4
Dominance_D	0.19	0.33	0.28	0.56	0.14	0.25	0.10	0.16	1.00	0.14	0.25
Simpson_1-D	0.81	0.67	0.72	0.44	0.86	0.75	0.90	0.84	0.00	0.86	0.75
Shannon_H	1.73	1.10	1.33	0.64	2.13	1.39	2.40	1.91	0.00	2.07	1.39
Margalef	2.40	1.82	1.86	0.91	3.25	2.16	4.06	2.89	0.00	2.82	2.16
Fisher_alpha	10.91	0.00	9.28	2.62	11.41	0.00	27.85	26.78	0.00	7.75	0.00
Berger-Parker	0.25	0.33	0.40	0.67	0.25	0.25	0.20	0.25	1.00	0.24	0.25
chao1	12.56	6.50	9.13	3.46	34.48	10.40	61.00	25.05	2.34	12.55	10.25

Table 7. The number of collected individuals and the number of traps per locality; the ratio of the number of collected bees and the number of traps.

Locality	Number of bees	Number of traps	Number of bees/traps
Trnovo, Crna Rijeka-Željeznica	1	2	0.5
Trnovo, Hojta-Presjenica	5	6	0.8
Gornjebosansko, Gornja Misoča	4	8	0.5
Igmansko, Hadžići Zujevina	4	9	0.4
Skakavac, Vučja Luka, Vogošća-Bulozi	15	28	0.7
Trebević	8	17	0.5
Bijambare	8	21	0.4
Gornjebosansko, Gornja Ljubina	3	22	0.1
Trnovo, Gornja Rakitnica	17	25	0.7
Gornjebosansko, Kaljina-Bioštica	3	28	0.1
Igman	16	93	0.2
Total	84	259	

Table 8. Multivariate linear regression analysis between habitat heterogeneity vs the number of bee species, the number of collected bees and Shannon - H for bee diversity.

Variable	Slope	Error	Intercept	Error	r	p
Number of bee species	-0.43	2.67	6.02	2.62	-0.05	0.87
Number of bees	-1.15	4.37	8.66	4.30	-0.09	0.80
Bee diversity indicated by Shannon – H	0.39	0.52	1.11	0.51	0.24	0.47

do not correspond to earlier assumptions about the positive connection between the heterogeneity of forest habitats and the number of insects and bees. This study shows that bark beetle slit traps can be used for monitoring of *Megachile* and *Osmia* genera within woodland communities.

Author Contributions

OM, AV, MD and SI conceived and designed the research, DP collected sample for analysis, DK carried out the sample separation,

AV performed taxonomic analysis, DP and AV processed the data and performed the statistical analysis, OM, AV, MD and SI secured the research funding, supervised the research and helped to draft the manuscript, AV, DK, OM and DP wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Experimental Study of Thermal Resistance Values of Natural Fiber Insulating Materials under Different Mean Temperatures

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ABSTRACT

The purpose of this paper is to experimentally study the thermal resistance (RSI value) of building insulation materials made mainly from natural fiber. Natural fibrous materials or renewable resources and their reinforcement composites are currently being used in building and construction as a potential solution to significantly reduce thermal load and energy consumption. The RSI value is used in describing the thermal efficiency of insulating material and in an analysis of heat transfer through the structural components of a building (such as walls, roofs, and windows) under steady-state conditions. In this study, the thermal resistance values of several samples made from coir fiber, rice straw fiber, energy reed fiber, and coconut wood were calculated from the thermal conductivity which was measured at mean temperature of 20°C, using the heat flow apparatus. The lowest RSI value was recorded in the phenol-formaldehyde polymer composites reinforced by rice straw fiber ($0.115 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$) and coir fiber ($0.128 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$) due to the relative thinness of the tested samples (8 and 12 mm). However, these samples can be used as an additional layer in multi-layered assemblies because of their low thermal conductivity value. The highest RSI value was reported on the binderless coir fiber panel ($0.909 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$) at the thickness of 50 mm. Another investigation examined the relationship between RSI value and mean temperature to observe the influence of variations of ambient temperature on the heat resistivity of building insulation materials. Practical data showed the decreased linear proportion between thermal resistance and specific mean temperatures increased from 0 to 40°C. It is apparent that an increase in the interior and exterior temperature of a building significantly influences the thermal resistance of its insulation materials. Based on the experimental study, once the thermal conductivity coefficient of each sample was determined, the calculated RSI value was a valuable parameter to evaluate the thermal resistant effectiveness of a multi-layered installation, which allows us to investigate practically the effect of the thickness of additional layers from different insulating materials used in building envelopes.

Keywords: coir fiber; polymer biocomposites; reed fiber; rice straw fiber; thermal conductivity; thermal resistance

INTRODUCTION

In the context of development in green technology and sustainable development, enhancing the energy efficiency in buildings and constructions, as well as reducing the global gas emissions and the dependence on traditional resources, natural fiber or plant-based fiber materials are used as a possible solution to meet these requirements. The outstanding advantages of insulation materials derived from natural resources are the qualities of being renewable, lightweight, environmentally friendly, and biodegradable. In

addition, natural fiber-reinforced polymer composites have shown better mechanical capabilities, physical properties, and thermal performance. Therefore, they can be used as a potential replacement for synthetic fiber-fabricated composites.

Common cellulosic fiber used as reinforcement in building insulating materials were extracted from different parts of the plant such as stem (i.e. flax, jute, hemp), leaf (i.e. banana, pineapple), seed (i.e. coir, cotton), stalk (i.e. rice straw, oil palm), and grass (bamboo, bagasse). Natural fibrous materials are generally comprised of cellulose (30–80%),

hemicellulose (5–40%), and lignin (5–20%) (Jawaid and Khalil 2011). The chemical compounds of coir, rice straw, and reed fiber are shown in Table 1. They are plant-based resources that are the raw materials used to manufacture insulating materials for building envelopes due to the low density of their fibers, high strength, and high heat retardant qualities. Besides, some products made from these fibers such as insulation boards or biocomposites were reported to have low thermal conductivity. For instance, thermal conductivity of rectangular straw-bale was found to be $0.066 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ at a density of $75 \text{ kg}\cdot\text{m}^{-3}$ (Conti et al. 2016), or ranged from 0.051 to $0.072 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ at a density of $80 \text{ kg}\cdot\text{m}^{-3}$ (Douzane et al. 2016). Thermal conductivity of giant reed particleboard was recorded from 0.059 to $0.094 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ at the density of $567\text{--}843 \text{ kg}\cdot\text{m}^{-3}$ (Andreu-Rodriguez et al. 2013).

The most effective approach to evaluate the heat resistance of an insulating material and the heat loss of a structure is through its thermal resistance (RSI value, $\text{m}^2\cdot\text{K}\cdot\text{W}^{-1}$). The higher the RSI value the better ability of insulation materials is to resist the flow of heat. It is measured based on the standard ISO 8301:1991/Amd 1:2022 (ISO 1991) Thermal insulation – Determination of steady-state thermal resistance and related properties — Heat flow meter apparatus — Amendment 1 or EN 12667:2001 (European Standardization Committee 2001) Thermal performance of building materials and products. Determination of thermal resistance by means of guarded hot plate and heat flow meter methods. Generally, the RSI value depends on the type of insulation, consisting of the material, thickness, and density (Peng and Wu 2008). The earliest experiments were conducted to investigate the thermal conductivity (λ -value) of some potential insulation materials made from fibers, and their thermal resistance value was determined through the thickness of the samples. Thermal conductivity of wood waste ranged from 0.048 to $0.055 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$, which was close to those of organic insulation materials such as jute ($0.038\text{--}0.055 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$), and the highest RSI value at a mean temperature of 30°C was $1.13 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$, showing that these materials can be used as good insulating materials (Cetiner and Shea 2018). The equivalent thermal resistance values and thermal conductivity of cardboard panels were also investigated (Čekon et al. 2017). The results showed that the cardboard-based materials can be an attractive replacement to commonly used thermal insulating materials due to the lowest λ -value ($0.0495 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$) and highest RSI value (0.687

$\text{m}^2\cdot\text{K}\cdot\text{W}^{-1}$) at a mean temperature of 20°C . Another study on binderless coconut husk and bagasse insulation boards (Panyakaew and Fotios 2011) reported thermal conductivity values ranging from 0.046 to $0.068 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ and from 0.049 to $0.055 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$, respectively. Since the thickness of both samples was 25 mm , the highest calculated RSI value was $0.54 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$. The thermal conductivity values of samples made from sugarcane bagasse fiber and polyvinyl alcohol as a binder ranged between 0.034 and $0.042 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$. In that case, the highest RSI value calculated at the thickness of 20 mm was $0.59 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$ (Mehrzad et al. 2022). According to the experimental data of these studies, the thermal resistance values of natural fiber-based materials were determined from their thermal conductivity values at room temperature (from 20 to 25°C), and the data was notably reported as higher than $0.5 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$. This is compatible with the EN 12667 standard which states that the insulation materials have the expected thermal resistance equals to or higher than $0.5 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$ and that thermal conductivity equal to or lower than $0.06 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ can be considered as a good thermal insulator for building application.

The present work investigates the thermal resistance of biocomposite insulation boards reinforced with rice straw and energy reed fiber, laminated timber panels made from coconut wood, and binderless insulation panels made from coir fiber. The thermal conductivity values were primarily measured at a mean temperature of 20°C , after which the RSI value was calculated through the thickness of tested samples. The temperature dependence of thermal resistance was practically investigated as the operating temperature increased from -5 to 45°C between the hot and cold sides. Finally, the relationship between the RSI value and the mean temperatures was also examined to observe the influence of operating temperatures on the heat resistance capacity of building insulation materials.

MATERIALS AND METHODS

Materials

The raw fiber materials used in this research are coir, rice straw, and energy reed fibers currently available in many tropical countries. Coir materials (*Cocos nucifera* L.) were collected from Vietnam, rice straw from the local rice fields in Hungary, and the energy reeds (*Miscanthus* spp.)

Table 1. Chemical compositions of coir, rice straw, and energy reed fiber.

Fiber	Chemical compounds (%)				Reference
	Cellulose	Hemicellulose	Lignin	Ash	
Coir	32-43	15-25	40-45	2.22	(Pillai and Vasudev 2001)
	36.6	37	22.2	1.9	(Kochova et al. 2020)
Rice straw	38	25	12	19.2	(Yokoyama and Matsumura 2008)
	36.5	33.8	12.3	13.3	(Sun et al. 2000)
Energy reed	47.95	-	24.85	2.9	(Abou-zeid et al. 2015)
	50.3	21.7	15	4	(Wahid et al. 2015)

from a company located in Lengyeltóti, Hungary. The raw materials were pre-treated with NaOH 5% for removing impurities present in the raw plant material. Later, they were washed with water to eliminate the excess pollutant particles and then dried in the oven. Finally, they were defibrated using a defibrating machine and were sieved for ensuring homogeneous fiber dimensions before composite production. Coconut wood for manufacturing cross-laminated timber panels was collected in Thailand and the samples were prepared at the laboratory of Center of Excellence in Wood and Biomaterials at Walailak University.

Sample Preparation

Figure 1 shows the samples used for the thermal test, namely rice straw and reed fiber reinforced phenol formaldehyde (PF) biocomposites (REPF), coir fiber reinforced phenol formaldehyde polymer biocomposites (CFPF), binderless coir fiber panel (BCFP), and cross laminated timber made with coconut wood panel (CTCP). The REPF and CFPF were manufactured by mixing the pre-treated fiber with the phenolic resin, the mixture was compressed using the hot-pressing technology at different pressure (7.1 MPa, 4.7 MPa, and 3.2 MPa) and the temperature was set at 135°C (Hasan et al. 2021a, Hasan et al. 2021b). Three samples were used for thermal conductivity test.

For binderless coir fiber panel, 105 g of fibers were prepared and compressed manually in the mould with 250 × 250 mm to shape the panel. The panel was then surrounded by the polystyrene holder to ensure the one-dimensional heat flow over the metered area. Three CTCP specimens were prepared as seen in Figure 1(d). All tested samples were cured for 1 day in ambient laboratory conditions.

Thermal conductivity value (λ -value) was determined in accordance with standard test method for steady-stated heat transfer by means of heat flow meter apparatus (according to standards: EN 12667:2001 (European Standardization Committee 2001), and ISO 8301:1991 (ISO 1991)). The use of the heat flow meter (HFM) method to measure the steady-state heat transfer through flat slab specimens and the calculation of the thermal conductivity by the heat transfer (q) was measured by the heat flux sensor (size = 120 × 120 mm, an accuracy of 0.1 W·m⁻²) inserted at the middle of the heating plate. For the thermal test instruments, heat flow

apparatus at the Laboratory of the Department for Timber Architecture at University of Sopron (for large sample sizes up to 600 × 600 mm, but not less than 250 × 250 mm) was used. Thermal conductivity coefficient (k) was calculated using the equation (1)

$$k = -\frac{q}{dT/dx} \quad (1)$$

where q is the heat flow rate (W·m⁻¹) and dT/dx is temperature gradient (K·m⁻¹). Thermal resistance (noted as R , in SI unit m²·K·W⁻¹), was calculated from the formula (2)

$$R = \frac{d}{k} \quad (2)$$

where d is the thickness of sample (m), and k is the thermal conductivity (W·m⁻¹·K⁻¹).

For temperature dependence, the thermal resistance values were determined through the thickness of tested samples and the coefficient of thermal conductivities which were measured at five specific mean temperatures incremented by 10°C from 0 to 40°C, specifically shown in Table 2.

RESULTS AND DISCUSSION

Thermal Resistance

Thermal conductivity values of the tested samples measured at a mean temperature of 20°C are shown in Figure 2, and thermal resistance (RSI value) calculated regarding

Table 2. Operating temperature between cold and hot sides.

Mean temperature (°C)	Cold plate (°C)	Hot plate (°C)
0	-5	5
10	5	15
20	15	25
30	25	35
40	35	45

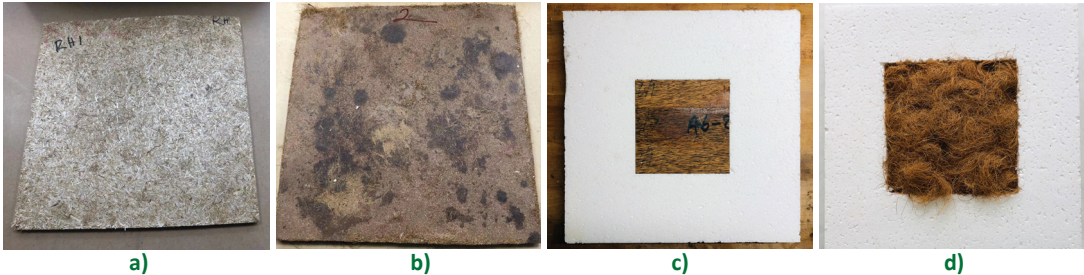


Figure 1. Tested samples: (a) rice straw and reed fiber reinforced PF biocomposites (REPF); (b) coir fiber reinforced PF biocomposites (CFPF); (c) binderless coir fiber panel (BCFP); (d) cross-laminated timber made with coconut wood panel (CTCP).

the thickness is shown in Table 3. Rice straw and energy reed fiber reinforced phenol formaldehyde (PF) polymer composites (REPF) showed the lowest value ($0.115 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$) at the thickness of 12 mm, followed by the RSI value of coir fiber reinforced phenol formaldehyde biocomposites (CFPF) which amounted to approximately $0.128\text{--}0.130 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$ at the thickness of 8 mm. The low thermal resistance came from the high thermal conductivity values measured and the high conductivity of phenolic resin used in the composites (the thermal conductivity coefficient of phenolic resins was 0.29 to $0.32 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ and its moisture content was nearly 34%, (Yang 2007)). While the thermal resistance of these samples was less than $0.2 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$, the CFPF can be seen as a more efficient insulation material than RFPR due to the lower thermal conductivity values. Moreover, the effect of PF resin in the whole composite was not significant since the used amount was only 10% in the composite production. Additionally, these composites can be used as an additional layer in multi-layered assemblies. For example, Yuan investigated the impact of insulation type and thickness on the thermal performance of a multi-layered wall structure (Yuan 2018). Without insulation material, the total RSI value (R_{eff}) was $1.26 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$ based on the following equation

$$R_{\text{eff}}=R_e+\sum (d_i/\lambda_i)+R_i \tag{3}$$

where R_e and R_i are the external wall surface resistance and internal wall surface resistance ($\text{m}^2\cdot\text{K}\cdot\text{W}^{-1}$), d is the thickness of materials' layer (mm), and λ is the thermal conductivity ($\text{W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$). Since the CFPF and REPF were employed as insulation layers, the total RSI value showed a slight increase, specifically, $1.4 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$ and $1.395 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$, respectively. Consequently, it would improve the heat resistance capacity of the building. The binderless coir fiber panels (BCFP) showed the highest thermal resistance value at 50 mm thickness, which was close to the value of sprayed polyurethane thermal insulation at 30 mm thickness ($1.071 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$ (Echarri et al. 2017)). The thermal conductivity of CTCP revealed the highest values since coconut wood conducted more heat than other materials, especially at higher density. Nevertheless, they showed higher thermal resistance than CFPF and REPF samples due to their greater thickness. Although thermal resistance of cross-laminated timber made with coconut wood panels (CTCP) showed a low value (lower than $0.5 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$, compared to BCFB), they had a higher RSI value than the maximum value of laminated timber made with *Pinus Oocarpa* Schiede ex Schlechtendahl and *Coffea Arabica* L. waste ($0.115 \text{ m}^2\cdot\text{K}\cdot\text{W}^{-1}$ at 30 mm thickness (Furtini et al. 2021)). Therefore, the CTCP may be used as insulation materials for building applications.

As it can be observed from the results, the thermal resistance value of an insulation material significantly varies depending on its type and thickness. Generally, thicker materials have higher thermal resistance values than thinner materials of the same type. However, the present study mainly investigated the thermal resistance values of four types of insulation materials with different thickness and their changes regarding the effect of mean temperatures. This can be considered in further studies that will examine the thermal performance of the multi-layered insulation materials at different thicknesses under the influence of variations of ambient temperature and relative humidity.

Relationship between Thermal Resistance Values and Mean Temperatures (R-T Relationship)

The influence of mean temperature in thermal resistance values of binderless coir fiber panels (BCFP) and cross-laminated timber coconut wood panel specimens (CTCP) is shown in Figure 3 and the specific values are shown in Table 4. As shown in the Figure 3, the RSI value of these samples decreased with increased mean temperature. It is obvious that higher temperatures always revealed higher thermal conductivity and because the thermal resistance is inversely proportional to its conductivity, increased thermal conductivity led to a decreased RSI value. On the other hand, it has been also reported that the thermal resistance values of CTCP decreased since the bulk density increased

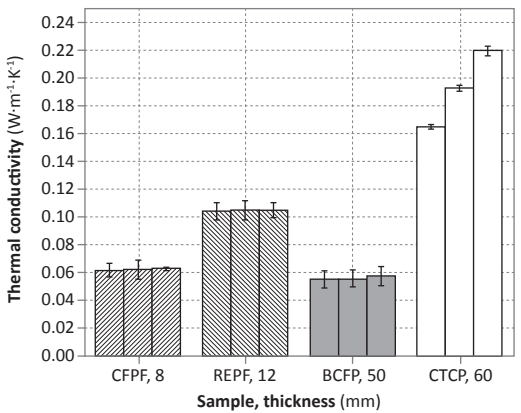


Figure 2. Thermal conductivity values and standard deviation of coir fiber reinforced phenol formaldehyde polymer biocomposites (CFPF), rice straw and reed fiber reinforced phenol formaldehyde biocomposites (REPF), binderless coir fiber panel (BCFP) and cross laminated timber made with coconut wood panel (CTCP) regarding the thickness, measured at mean temperature of 20°C.

Table 3. Thermal resistance values calculated according to equation (2).

Sample	Thickness (mm)	Thermal resistance ($\text{m}^2\cdot\text{K}\cdot\text{W}^{-1}$)
CFPF	8	0.128
		0.129
		0.130
REPF	12	0.116
		0.115
		0.115
BCFP	50	0.909
		0.898
		0.873
CTCP	60	0.365
		0.311
		0.273

from 678 to 828 kg·m⁻³. Having the same thickness, high-density wood conducts more heat than low-density wood due to the high heat conduction at a higher solid substance and the reduction of porosity at high density. As a result, the thermal resistance capacity of CTCP decreased relatively. For BCFP samples, it has been also shown that the RSI value decreased significantly regarding the increased density. This result was similar to the relationship between thermal resistance value of sugarcane bagasse waste fibers specimens (calculated from Eq. (2)) and the bulk density (Mehrzad et al. 2022). Specifically, the RSI value of bagasse fiber samples decreased from 0.588 to 0.476 m²·K·W⁻¹, while density increased from 100 to 200 kg·m⁻³ at a thickness of 20 mm.

It is essential to examine the heat resistance of any insulation materials due to their crucial role in determining the thermal performance of building envelopes. Undoubtedly, the thermal resistance value is the main key to evaluating the efficiency of insulation material to resist heat flow. It is calculated from the thickness divided by the thermal conductivity of the insulation. Because the λ-value is affected strongly by mean temperature and relative humidity (Le and Pásztor 2021), then so is the RSI value. According to the experimental data, the changes in

thermal resistance values regarding the increased mean temperature were presented as a linear reduction with a high R² value (see Table 4), demonstrating the great influence of temperature on the thermal resistant capacity. In fact, the molecule transfer will become faster with the increase of the operating temperatures, leading to an increase in heat flux across the thickness of insulation materials, and therefore decreasing the RSI value. The linear relationship can be explained by the sole heat conduction mechanism in the steady-state condition of the experiments.

CONCLUSIONS

This paper investigated the thermal resistance values of samples made from natural fiber materials. The main goal of the experiments was to determine thermal resistance values in different thicknesses at room temperature and the dependence of the R-value on operating temperatures. According to the thermal conductivity results, most of these samples are potential thermal insulation materials used in building envelopes. Only cross-laminated timber made with coconut wood was not suitable. The calculated R-values of CFPF and REPF showed that they can be used in the multi-

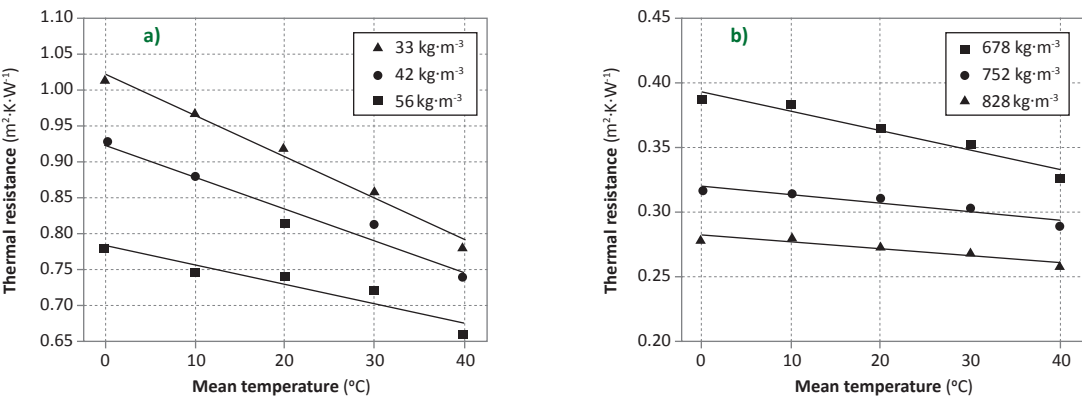


Figure 3. Relationship between thermal resistance values and R² value: (a) binderless coir fiber panels (BCFP) (regarding density); (b) cross-laminated timber coconut wood panels (CTCP) (regarding density).

Table 4. The thermal resistance of binderless coir fiber panels (BCFP) and cross-laminated timber coconut wood panels (CTCP) regarding the increased mean temperatures.

Sample	Thermal resistance (m ² ·K·W ⁻¹)					R-T relationship	R ²
	Temperature (°C)						
	0	10	20	30	40		
BCFP	0.7796	0.7481	0.7407	0.7229	0.6579	-0.0027 X T + 0.7836	0.89
	0.9281	0.8785	0.8163	0.8122	0.7421	-0.0044 X T + 0.9231	0.96
	1.0142	0.9671	0.9174	0.8591	0.7813	-0.0057 X T + 1.0226	0.99
CTCP	0.3859	0.3839	0.4130	0.3517	0.3272	-0.0015 X T + 0.3926	0.94
	0.3176	0.3146	0.3650	0.3041	0.2897	-0.0007 X T + 0.3207	0.92
	0.2801	0.2808	0.2733	0.2683	0.2592	-0.0005 X T + 0.2832	0.92

layered installation. The thickness factor made a significant difference in the thermal resistance values. The research has also shown that the thermal resistance values decreased with increased mean temperature, and their relationship is presented as a possible linear correlation.

These findings contribute in several ways to our understanding of thermal resistance values of natural fiber-based insulation materials and provide a basis for further investigation on multi-layered insulation materials. As expected, natural fiber has proved to be an effective resource used as raw material in reinforcement polymer composites and has been valued as an essential replacement for traditional insulation materials in the future.

Author Contributions

DHAL generated the idea, carried out the sample preparation; conducted the experiment, analysed the data, prepared the first draft of the manuscript. ZP is responsible for the research project and revision.

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Conflicts of Interest

The authors declare no conflict of interest.

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Preliminary Work on Generative Seedling Production and Clone Selection of European Black Poplar (*Populus nigra* L.)

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ABSTRACT

European black poplar (*Populus nigra* L.) is a pioneer species that belongs to the *Salicaceae* family and occurs in riparian ecosystems. It is one of the most endangered forest species in its entire distribution area. In Croatia, black poplars are considered an economically important forest species, but mostly clones originate from crossing combinations with American and European black poplar (*Populus × canadensis* Moench), while a small number of clones are native black poplar. Studies on native black poplar are quite rare and the generative propagation has not been used. The aim of this study was to gain knowledge on the production of high-quality black poplar seedlings and to carry out the selection of genotypes with the aim of establishing a base for future breeding. The female black poplar tree was selected on phenotypic characteristics in the area of Forest Administration Osijek, Forest Office Valpovo. The tree was cut down in April 2019, and branches with half-open seed capsules on catkins were collected. The branches were transferred to the Croatian Forest Research Institute's greenhouse, where the catkins opened under the influence of the high temperature. Sowing was done in different substrates to test their effectiveness. Black poplar seedlings were selected and transplanted with regard to development and height growth. The results showed differences in height growth between plants sown in two different substrates and the occurrence of fungal diseases only on plants sown in pure sand. With subsequent multiple propagation using cuttings and selection by genotype, it is expected that it will be possible to identify several clones of native black poplar that will be introduced for use in forestry in Croatia. The use of quality plants grown from seeds will increase the genetic diversity and preserve the native black poplar gene pool.

Keywords: black poplar; seeds; gene pool; substrates; selection

INTRODUCTION

European black poplar (*Populus nigra* L.) is a species belonging to the *Salicaceae* family. It belongs to extremely halophilic species, which as pioneer species inhabit exclusively areas without trees (Kajba and Romanic 2002). It has a wide distribution area (Zsuffa 1974) and is a dioecious species that flowers before leafing and is pollinated by wind. Individual trees can grow up to 400 years (Popivshchy et al. 1997). Black poplar is characterized by a great diversity of populations, from isolated tree as well as pure stands to mixed stands (Lefèvre et al. 1998). It mainly grows in wet floodplains along rivers. In nature, it is mainly propagated by seeds, while the vegetative

method is most often used for economic purposes. A single female black poplar tree can annually produce thousands or even millions of non-dormant small seeds (about 1 mm long), with high germination (>90%) (Van Splunder et al. 1995, Karrenberg and Suter 2003), but short-term viability (Karrenberg and Suter 2003), which are dispersed by wind and water over a large distance. Sexual regeneration with seeds increases genetic diversity, population resistance to environmental changes and various diseases (Lefèvre et al. 2001, Barsoum et al. 2004). Unfortunately, European black poplar is one of the most endangered species in Europe (Wójkiewicz et al. 2021). The main reasons are the loss of natural alluvial habitat, especially the sandy and gravelly river

coast that are necessary for the success of the seeds, and competition and introgression with non-native species from the genus *Populus* sp. (Fossati et al. 2004, Vanden Broeck et al. 2005, Ziegenhagen et al. 2008, Meyer et al. 2018). In riparian areas of Croatia, mainly planted clones of poplars are *Populus* × *canadensis* Moench and *Populus deltoides* Bartr. ex Marsh., which endangers *Populus nigra* L. gene pool. Restoration and protection of floodplain forests is one of the main priorities in biodiversity conservation and adaptation strategies related to climate change (e.g. EU Biodiversity Strategy 2030, EU Floods Directive 2007/60/EC). Floodplain forests are a hotbed of biodiversity and provide a number of ecosystem benefits including flood protection, erosion, soil thermal regulation with tree canopy cover, and water quality protection (Van Looy et al. 2013).

European black poplar is one of the main species of the floodplain ecosystem that belongs to the economically important forest species in Croatia. In recent years, there has been a serious problem of survival, primarily due to the loss of habitat and the impossibility of natural regeneration. Therefore, this is the reason why it was decided to start with works that include the production of seedlings from seeds (by generative reproduction). According to our knowledge, there has been no other such method of producing European black poplar seedlings in Croatia. Also, there is still no protocol for the production of quality seedlings that would include good practices from the collection of mature branches from the mother trees, through the manipulation of seeds to the growing quality seedlings in the nursery. Due to the importance of this species in the context of the preservation of the biodiversity of fluvial ecosystems, as well as its economic value, it is necessary to establish the protocol in the future.

The main goal of this work is to gain practical experience in the production of quality black poplar seedlings as the first step in this process. Other objectives are: i) to compare the

height development of seedlings in two sowing substrates and ii) to perform an initial selection of genotypes with the aim of establishing a base for breeding.

MATERIALS AND METHODS

Sampling Area of Reproductive Material

The largest areas of European black poplar in the Republic of Croatia are located in its eastern part, Slavonia and Baranja, along the Drava and Danube rivers. Although its total area has been decreasing and dispersing over the years, larger natural stands can still be found in this area. This is supported by the existence of the only black poplar forest seed object in Croatia in the category 'selected', register number HR-POP-SS-111/117, which is located in the area of Forest Office Osijek, right next to the estuary of the Drava and the Danube.

This research was carried out in the area of Forest Administration Osijek, in the area of the Forest Office Valpovo, on the banks of the Drava river in a natural stand of European black poplar was found, which is located in the management unit Valpovačke podravske šume, department/section 4b, on area of 9.35 ha. There are no cultures and plantations of other species from the genus *Populus* sp. nearby, so unwanted pollination could not occur, which brings us to conclusion that this is pure stand.

Sampling Method

In the selected natural stand, based on good phenotypic appearance and health status, a female tree was selected in April 2019. The selected tree was fallen because it had already been marked previously, i.e., scheduled for felling in the same year. Ten 1 m long top branches with catkins (early ripening seed capsules) were cut from the felled tree to obtain seed material (Figure 1).



Figure 1. Collected branches with catkins containing black poplar seeds.

Method of Obtaining Seed Material

The collected branches with catkins were transported to the Croatian Forest Research Institute on the same day, shortened to a length of half a meter and stored in a greenhouse. There were 4-7 catkins with seeds on each sampled branch. The branches were put in plastic containers with water and covered with breathable cotton material so that the seeds would not disperse after opening of the catkins. In a period of 24 hours under the influence of high temperature in the greenhouse (15-34°C) and sufficient amount of air moisture, the catkins with the seeds opened and the seeds came out (Figure 2). There was an average of 15 seeds per capsule, and about 15,000 seeds were obtained from this quantity of sampled catkins. Due to the large amount of seeds obtained, 1/3 of the total amount of seeds were used (approx. 5,000 seeds). The seeds of the black poplar are covered with a white silky hair giving a fluffy a cotton like layer, which the wind blows over long distances (Barsoum 2001). For better sowing efficiency the cotton like layer had to be separated from the seeds. This was done mechanically, i.e. by rubbing on a metal sieve with 2.38x2.38 mm holes.

Sowing Method

Black poplar seeds are characterized by good germination and high vitality. However, the minimal energy reserves of the small seeds limit their longevity to a period of only a few days or weeks (Moss 1938, Muller et al. 1982, Johnson 1994, Van Splunder et al. 1995, Braatne et al. 1996, Karrenberg and Suter 2003), preventing long-term seed storage stock in the seed bank.

For these reasons, sowing was started within 24 hours after obtaining clean seeds. Two types of substrates were used for sowing. The first type was sterilized fine-grained sand, which is used for seed germination, and the second

type was a mixture of Drava sand (granulation 0.2 mm) and peat (DURPETA PROFI MIX 1a, pH 5.5-6.5). The seeds were sown in 59x39x15 cm containers. When sowing, the seeds were evenly distributed and covered with a thin layer of the substrate. Since it is very important that the seeds have enough heat and moisture after sowing, this was achieved by sprinkling them twice a day. Sowing in different substrates was used for comparison, i.e., testing their effectiveness.

Further Seedlings Procedures

Germination of black poplar seeds occurs rapidly (usually within 24 hours), but the seeds must have sufficient moisture (Siegel and Brock 1990, Van Splunder et al. 1995). However, the seedlings do not tolerate shade, i.e. they are less competitive compared to natural vegetation (Gage and Cooper 2005). Therefore, the colonization of alluvial deposits and forming of young stoolbeds occurs during the retreat of floodwater. In addition to moisture, light is also important for successful germination.

With suitable conditions, i.e. enough moisture and light (heat in greenhouse conditions), the second day after sowing the seeds began to germinate. The radicle appeared first, then the cotyledons, which still had the seed coats on them (Figure 3).

Due to high humidity and high temperature in the greenhouse, fungal diseases (seedlings damping and common pinmould) appeared on about 1,500 seedlings only in pure sand substrate (Figure 4). After the appearance of fungal diseases, chemical treatment with Previcur Energy and Folicur EW 250 fungicides were applied in appropriate doses in pure sand.

With the appearance of the first leaves and the strengthening of the plants, the conditions were created for the first transplant into individual holes in 18 Bosnaplast plastic containers (32x21.5x18 cm, 33 holes in the block



Figure 2. Opened seed capsules on catkin from which black poplar seeds have emerged.



Figure 3. Seedlings of European black poplar.



Figure 4. The appearance of fungal diseases on black poplar seedlings.

(the volume of each hole is 220 cm³). When transplanting, plants that differed from the others were selected, i.e. all that had developed two leaves and reached an average height of 2 cm. A total of 499 plants were selected from the initial number, which was a rough estimate of 3,000 seedlings. From 5,000 sown seeds, about 3,000 seedlings were obtained, indicating a germination rate of 60%. Transplanting was done towards the end of June, before the summer heats. The containers were previously filled with a mixture of neutral peat (DURPETA PROFI MIX 1a) and Drava sand (granulation 0.2 mm) in a ratio of 250 l of peat and 50 l of Drava sand. The transplanted plants were moved from

the controlled greenhouse conditions to outdoor conditions in the nursery where they were regularly watered, and their health condition was monitored (Figure 5).

At the end of the vegetation period, in November, the second transplanting of plants was started. The plants were transplanted into individual, larger containers (21.5x10 cm (volume 2 l)). The containers were filled with a substrate that is a mixture of neutral peat and Drava sand (the same substrate as for the first transplant). During the second transplant, an additional selection of cultivated plants was performed, during which 323 plants were selected out of a total of 499 plants after the first selection. Plants that had



Figure 5. Black poplar plants in individual containers.

a height increase of at least 1 cm since the first transplant and plants that had developed more than two leaves were selected. The plants in the containers were arranged in the open germinator in the nursery of the Croatian Forest Research Institute, where they were watered and health condition was monitored on a regular basis.

Seedling Measurements and Data Processing

In November 2019 and 2020, during plant dormancy, plant heights were measured using a wooden meter with a precision of 0.5 cm. The heights were measured to determine the height difference between the plants depending on the substrate used when sowing the seeds, i.e. to determine the efficiency of the applied substrates, but also for the additional selection of individuals that showed the greatest height increase.

Student's t-test in MS Excel was used to compare the average heights of seedlings according to substrate type. For this statistical analysis, a significance level of 5% ($p < 0.05$) was considered statistically significant.

Selection of Genotypes

One of the basic methods in plant breeding is the selection of genotypes with regard to desired properties. In this research, using seed material taken from one mother tree, a large number of plants with relatively high genetic diversity were obtained (compared to seedlings obtained by vegetative propagation). After germination and until the first transplant, there were visible differences in the progress of plants that had the same conditions for growth. Selections were gradually made from the initial large number of plants, i.e. the most productive individuals were selected (as previously explained). The selection criteria were height growth and the development of the plant, i.e. the number of leaves. Therefore, in order to select the most productive plants at the end of the growing season, the plants that differed in height growth were gradually transplanted into pots with more soil and their progress was further monitored.

RESULTS

By measuring the height of seedlings grown in two different sowing substrates, and comparing them, a statistically significant difference was obtained. In 2019, the p -value was $3.0007e-18$, while in 2020 it was $1.3309e-06$.

The results shown on the Box-Whisker diagram (Figure 6) show the differences in the height of black poplar seedlings in different substrates in 2019 and 2020. The average height of seedlings in 2019 was 9.42 cm for the substrate "sand and peat" and 7.05 cm for the substrate "sand" (Table 1). In 2020, the average height of seedlings was 14.47 cm for the substrate "sand and peat" and 11.22 cm for the substrate "sand". Interquartile values show the range of data in 2019 from 7.5 to 11.5 cm in height for the sand + peat substrate, and from 5.5 to 8.0 cm for the sand substrate. In 2020, the interquartile values show a range of data from 9.87 to 17.0 cm in height for the sand + peat substrate, and from 7.0 to 14.62 cm for the sand substrate.

In addition to the results presented, where differences in height growth are visible, it is important to note that fungal diseases appeared only on plants that were sown in pure sand. Obtaining seeds using the applied method of branch collection and catkins manipulation proved a successful method, as sufficient quantities of branches with half-open catkins were collected, from which, after opening in the greenhouse, a quantity of about 15,000 black poplar seeds were separated by mechanical method of the "cotton layer" using a metal sieve. The success of seed germination was equal in both substrates, that is, there was no significant difference in germination between different substrates.

Table 1. Average height of black poplar seedlings in different substrate.

Substrate	N	Height in 2019 (cm)	Height in 2020 (cm)
Sand + peat	160	9.42±3.03	14.47±6.87
Sand	224	7.05±2.03	11.22±6.03

Note: Average ± standard deviation; N – the number

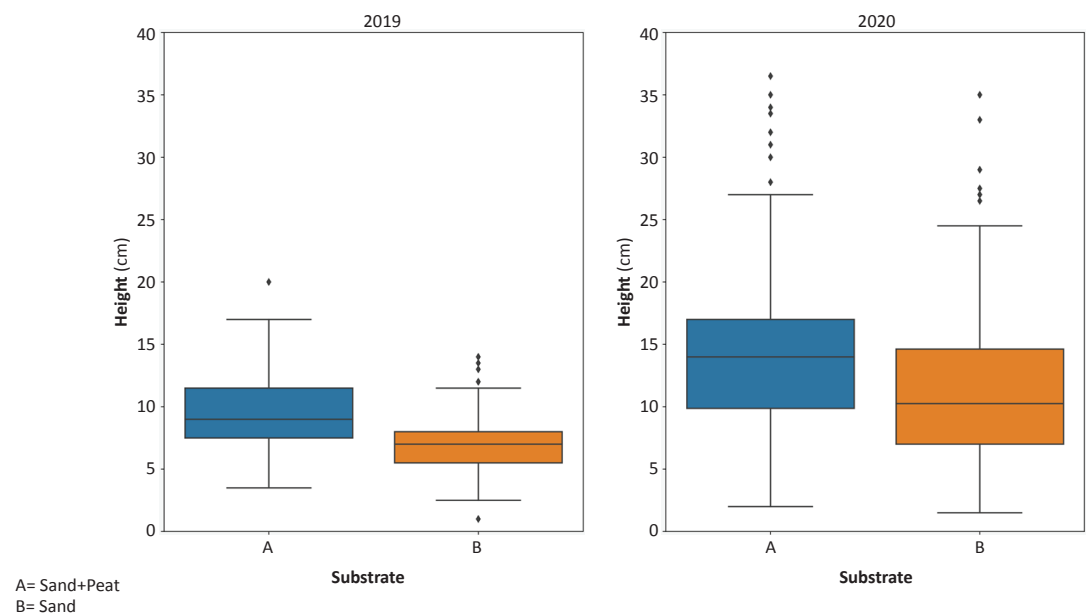


Figure 6. Height differences of black poplar seedlings in a different medium in the years 2019 and 2020.

Differences between seedlings and substrates were expressed later when plants needed more nutrients to thrive.

DISCUSSION

The methodology of collecting and sowing black poplar seeds and growing seedlings in nurseries needs to be improved, and respectively the best protocol for growing seedlings from seeds needs to be developed. Cultivation of seedlings from seeds is not common in Croatia, nor has it been practiced so far, due to the fact that productive clones are produced mainly from cuttings that are used either for the production of seedlings or renewal of cultures. These are American black poplar clones or hybrids of American and European black poplar that are unsuitable for use in protected natural areas. Collection of seed material, further manipulation of seeds and growing of plants in nurseries is a complex process that needs to be developed and adapted to specific conditions. Guilloy-Froget et al. (2002) emphasizes the importance of recognizing the time period of seed maturation, which in the case of black poplar lasts from 2 to 9 weeks, depending on the climatic conditions in the current year. This is important because it is necessary to determine the right time to collect mature seeds for sowing success. Also, it is necessary to observe the catkins on the female trees often and collect branches with catkins or just catkins before they fully open. In this research, the beginning of the opening of catkins was noticed in a timely manner. A time gap of only two or three days could have affected the complete opening of catkins and, in that case, the seeds would have been dispersed all around. Other options for collecting seed material are the climbing of certified

climbers on trees or the placement of seed catchers in the form of wooden cubes coated with glue under the crowns of trees, the so-called 'seed traps', (Cooper et al. 1999, Gage and Cooper 2005). The method of felling the selected tree is suitable if it is a single tree or a forest stand at the end of the rotation. This method proved to be successful because it was possible simply to collect a sufficient amount of seed material. However, it should be emphasized that the seed material should be collected from a larger number of trees and in different habitat conditions. This would achieve a significantly higher level of genetic diversity of the offspring and thus a better and wider basis for selection. Collected quivers can be left in paper bags at room temperature (Gladwin and Roelle 1998) or in a greenhouse at a higher temperature which affects earlier opening and releasing of the seeds. Before sowing, the seeds must be separated from the cottony layer using a metal sieve.

Germination of seeds and initial growing of seedlings in a greenhouse allows controlled homogeneous growth conditions for all plants. This work showed the disadvantages of using pure sand as a substrate for sowing and the subsequent cultivation of seedlings, while the mixed substrate (neutral peat and sand) proved to be significantly better, probably due to the higher content of nutrients, which made it possible to achieve an average higher height growth of seedlings in that substrate. For better plant development, the substrate mix for sowing or soil texture for subsequent poplar transplanting should have a sand content between 55 and 70% (Pinno and Bélanger 2009). Sher et al. (2002) states that poplar seedlings grow best in a substrate with more clay in the first year, and better in a substrate with more sand in the second year. Other studies mention that fast-growing species accumulate more biomass in mixed, heterogeneous

substrates than in homogeneous substrates such as pure sand (You et al. 2014, Luo et al. 2016). In certain works, it is stated that the use of a mixed substrate, which contains perlite, peat and sand, increases the height of seedlings and the number of leaves (Albaho et al. 2009). Although sand proved to be a good medium for germinating seeds, i.e. the seeds that were sown germinated very quickly, the plants later had little progress due to the lack of nutrients.

Another disadvantage of pure sand compared to mixed substrate is the occurrence of fungal diseases (seedlings' damping and common pinmould (*Mucor mucedo* L.)) because all seedlings that get symptoms of fungal infection were grown in pure sand. One of the possible explanations can be the property of sand to retain moisture and heat for a longer period, while in the case of a mixed substrate containing peat, there is a faster loss of moisture, and thus the substrate dries. In conditions of frequent irrigation, the substrate becomes saturated, and the high temperature creates ideal conditions for the appearance of fungal diseases. The occurrence of fungal diseases only on plants sown in pure sand leaves space for future research of this phenomenon.

The phase of transplanting plants represents another possibility for progress in future research. It was carried out twice during the year, which could have been the cause of the weaker progress of the seedlings due to stress during the growing season. The first transplanting was done in June, when the seedlings were transferred from the greenhouse to outdoor conditions under the shade and the second in November of the same year. In view of the above, it is recommended to use pots or containers with a larger capacity immediately after sowing, so that transplanting is done less often. Also, it can be recommended to transplant once a year (if necessary) during the non-vegetation period. The use of small pots or containers can negatively affect root health and stability (Lindström and Rune 1999, Cedamon et al. 2005). In addition, some authors list a number of suggestions for initial germination and later transplanting, such as keeping the seeds in clean water, then later keeping the seedlings in water for 8-10 days to allow their roots to elongate quickly. After that, such seedlings were immediately transplanted into natural conditions on different types of soil without previous cultivation in soil substrates (Hao et al. 2012).

It is also important to point out the problem of lack of quality water for watering seedlings in greenhouse conditions, because water from the water supply was mainly used. Such water has less nutrients, and it is also chlorinated, which is why it is not of the same quality as the water from lakes or rivers, which is usually used to irrigate plants in large nurseries. In addition, with the aim of better seedling growth, one should try to use a more natural substrate, such as alluvium, because research has shown that mycorrhizal associations can be important for better growth of black poplar (Lodge 1989).

The generative method of reproduction promotes the preservation of genetic diversity, which is necessary for adaptability, i.e. the long-term survival of a species in changing habitat conditions. Collecting seeds from more (min. 30) female trees significantly increases the possibility of finding and selecting the most productive genotypes for further production.

There is high importance to make diagnostic molecular genetic markers for characterization of taxonomic status. It should be used in all cases to confirm the taxonomic identity and non-hybrid nature of the selected trees as pure *Populus nigra* L. individuals.

Considering the trends in the protection of natural ecosystems, climate change, but also the tradition of productive forestry production, it is recommended to intensify the cultivation of generative seedlings in outdoor conditions, in nurseries specialized in poplar production. Such nurseries are located on high-quality land, in natural poplar habitats, and plants are irrigated with water from rivers or artificially raised lakes. In such conditions, plants from seeds achieve maximum growth and later it is possible to select genotypes and then propagate vegetatively and test them in clonal plantations in various habitat conditions.

CONCLUSIONS

Based on the conducted research, the following conclusions can be drawn:

- Average height growth was significantly higher in European black poplar plants grown in sand + peat substrate, compared to plants grown in pure sand. This can be explained by the fact that neutral peat mixed with pure sand enables better plant growth, as it contains nutrients that young plants need for growth. On the other hand, pure sand is not so rich in nutrients, and frequent irrigation leads to faster washing away of the already modest amount of nutrients. Although pure sand proved to be a good initial substrate for seed germination, as it allows for longer retention of moisture and breathability, it turned out that soon after emergence the plants must be transplanted into a soil substrate with more nutrients.
- Pure sand in conditions of frequent watering and high temperature favors the occurrence of fungal diseases, as they have all been recorded on plants sown in sand. A possible cause of such a phenomenon is longer retention of moisture and warmer micro-conditions of this type of substrate.
- The use of individual or larger containers enables more space for the development of the root system of seedlings. More frequent transplanting during the year also leads to plant stress, after which the plants progress more slowly, due to the adaptation to new conditions, and plant death is possible. Transplanting is best done only once a year and during the rest of the vegetation.
- Breeding of European black poplar seedlings from seeds is a rarely used method of reproduction and it needs to be improved, i.e. it is necessary to find the best protocol for breeding quality seedlings in our conditions.
- By using quality seedlings from seeds, the genetic diversity of planting forest reproductive material will be increased, the gene pool will be better preserved in long term, and with genetic testing and selection, we expect to identify one or more clones whose productivity will be competitive to the poplar's clones currently in use in forestry of the Republic of Croatia.

Author Contributions

ZV, SB2 and MI conceived the research, NZ processed the data and performed the statistical analysis, ZV and ML carried out the field work and measurements, SB2, AGM, ML, NZ, SB1, MI helped to draft the manuscript, ZV and SB2 wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Further Spread of *Corythucha arcuata* (Hemiptera; Tingidae) in Croatia

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ABSTRACT

Corythucha arcuata (Hemiptera; Tingidae), i.e. oak lace bug is an invasive alien species from North America that has rapidly spread in Europe. It was first reported in Croatia in 2013, and in the following years it has spread rapidly toward the west of the continental part of the country, infesting 200,000 ha of *Quercus robur* L. forest stands. Oak lace bug causes losses in chlorophyll, which has a negative influence on photosynthesis and transpiration activity, as well as on the health status of oak trees. We conducted our study on two sites in the Mediterranean region in Istria, Croatia, where infestation with oak lace bug has not been recorded. Results showed new records of oak lace bug in Istria. *Q. pubescens* Willd. is the dominant tree species in Sub-Mediterranean forests in Istria, so it will be interesting to follow the spread and preferences of oak lace bug for *Q. robur* and *Q. pubescens* in Istria, as well as in other coastal *Q. pubescens* and *Q. ilex* L. forests in Croatia. We assume that the negative influence of oak lace bug coupled with other biotic and abiotic stressors in the Mediterranean region will probably have some influence on the health status of oak trees.

Keywords: oak lace bug; Istria; alien invasive species; spread; *Quercus*

INTRODUCTION

Invasive alien species have been recognized as one of the main threats to biodiversity worldwide (Vilà et al. 2011) and the number of their introductions is constantly increasing (Seebens et al. 2017). *Corythucha arcuata* (Say) (Hemiptera; Tingidae), commonly known as the oak lace bug (OLB), is one of the recent introductions of alien species that has spread over Europe. It is native to North America, feeding on leaves of various North American oak species, with main hosts from genus *Quercus*. Other host species are species from genera *Castanea*, *Acer*, *Pyrus*, *Malus* and *Rosa* (Csóka et al. 2020a).

OLB was first reported in Italy in 2000 (Bernardinelli and Zandigiacomo 2000). It has since spread to other areas of Europe (Csóka et al. 2020a). Sap feeding by nymphs and adults causes losses in chlorophyll, which has negative influence on photosynthesis and transpiration activity (Nikolić et al. 2019). This impact could, together with other harmful biotic and abiotic factors, negatively influence the health of oak trees that are under additional stress from consequences of climate change (Acácio et al. 2017)

OLB was first recorded in Croatia in the pedunculate oak stands (*Quercus robur* L.) of Spačva forests in eastern part of Croatia in 2013 (Hrašovec et al. 2013). In the following years, OLB quickly spread to other parts of continental Croatia causing premature color change and yellowing of the leaves (OIKON 2019). Using MODIS satellite imagery, Kern et al. (2021) concluded that the damage caused by OLB is significant and persistent after the infestation has started.

Early detection of invasive alien species is important to facilitate various responses in controlling and/or monitoring of the species. Early detection of the pathways of introduction and speed of spread may provide important insights into the behavioral traits of invasive alien species in new environments that can be used for predicting its distribution and damages (de Groot et al. 2020).

OLB has been recorded only in continental part of Croatia. Sub-Mediterranean and Mediterranean forests in the coastal part of Croatia are important forest ecosystems that provide irreplaceable ecosystem services (Vukelić et al. 2018), and arrival of new alien species could pose a threat to these forest ecosystems that are under additional

pressure caused by climate change (MedECC 2020). The aim of our research is to record the occurrence of *C. arcuata* in Istrian Peninsula.

MATERIALS AND METHODS

Study Area

Our study was performed on two sites in Istria, Croatia (Figure 1). The distance between Site 1 and Site 2 is 5 km of clearance distance.

Site 1 (no.1 in Figure 1b) is an avenue lined with *Q. robur* trees around Istrian Thermal Resort (HTRS96 Lat.295105.14, Lon.5029778.49). In the avenue, 15 oak trees were inspected. Leaves were collected from the ground, up to the height of 2 m using telescopic pruning shears.

Site 2 (no.2 in Figure 1b) is in management unit Mirna (HTRS 96 Lat. 295723.5, Lon. 5025592.3) in central Istria, Croatia. The area of the management unit Mirna consists entirely of stands of *Q. robur* in the famous Motovun forest, which represents the last remnant of the autochthonous lowland flood forests called "longozas" in the river valleys

of the Mediterranean and Pontic coasts. The characteristic lowland terrain that rises slightly from west to east, together with the high level of underground water and occasional floods during spring and autumn and the influence of other synecological factors caused the appearance of lowland flood forests similar to those in Slavonia. The presence of many thermophilic elements (*Ruscus aculeatus* L., *Asparagus tenuifolius* Lam., *Arum italicum* Mill. and others) clearly distinguishes the Motovun forest from the related Slavonian flood forests (*Genisto elatae-Quercetum roboris*, *Leucojo aestivi-Fraxinetum angustifoliae*). A recent study (Vukelić et al. 2018) has classified mixed forests of hardwood broadleaved trees along the Mirna River in the association *Asparago-tenuifolii-Quercetum roboris*, which also includes Site. The second site covered the area of 13.30 ha. Oak trees were chosen using Python script for n number of plots in QGIS and for extracting its coordinates. In the field, using a hand-held GPS receiver, we have searched for the nearest oak tree to the plot extracted in QGIS. In total, 30 oak trees were chosen. The leaves were collected from the lower part of the tree and from the top of the canopy using big shots.

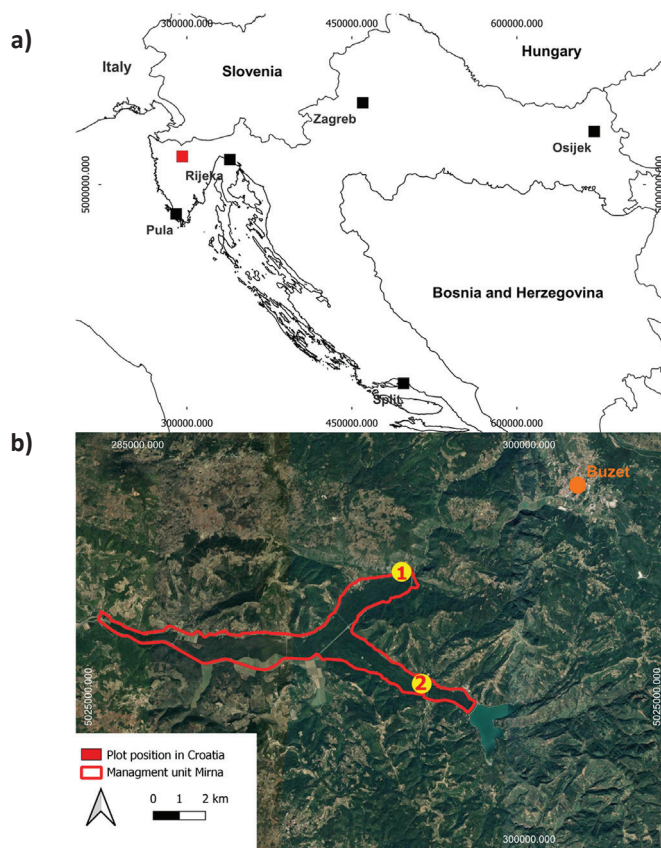


Figure 1. Location of plot sites in: **a)** Croatia (red mark); **b)** management unit Mirna.

Collecting Method

From each tree on both sites (15 trees on Site 1 and 30 trees on Site 2), 50 leaves from the north, south-east and south-west side were collected. Each collected leaf has been visually inspected for the presence of OLB. The leaves have been stored in sealed plastic bags and taken to entomological laboratory of the Croatian Forest Research Institute for closer inspection and morphological identification.

Identification

The collected egg masses and adults were identified in the Laboratory for Entomological Analysis of the Croatian Forest Research Institute according to Horn et al. (1979) using Olympus SZX7 stereomicroscope. The samples were stored in the freezer of the Laboratory for further analyses.

RESULTS

The sampling on Site 1 - Istria Thermal Resort resulted in the collection of leaves infested with OLB. On the leaves larvae and adults were present (Figure 2a, 2bb) and morphologically confirmed in the morphological laboratory

analysis. All 15 sampled trees were infested with *-C. arcuata*. On Site 2, out of 30 sampled oak trees, only one tree was infested with *C. arcuata* (HTRS96 Lat. 295950.221, Lon. 5025515.762). Egg masses and adults have been found on three (3) leaves on the northern part of the canopy (Figure 2c, 2d). On all other inspected trees on Site 2 egg masses, larvae or adults of OLB have not been found.

DISCUSSION

OLB was first recorded in the continental region of Croatia in 2013 (Hrašovec et al. 2013). In the same year (2013) this pest has been recorded in neighbouring Hungary (Csóka et al. 2013) and Serbia (Pap et al. 2015), and in the following years in Slovenia (Jurc and Jurc 2017) and Bosnia and Herzegovina (Dautbašić et al. 2018). The most recent finding of this pest in Europe was in the Czech Republic in 2020 (Csóka et al. 2020b) Since its discovery in Croatia, OLB has spread quickly to all continental oak forests, infesting over 200,000 hectares. However, it has not been detected in the Sub-Mediterranean or Mediterranean forests of Croatia (IPP 2021).

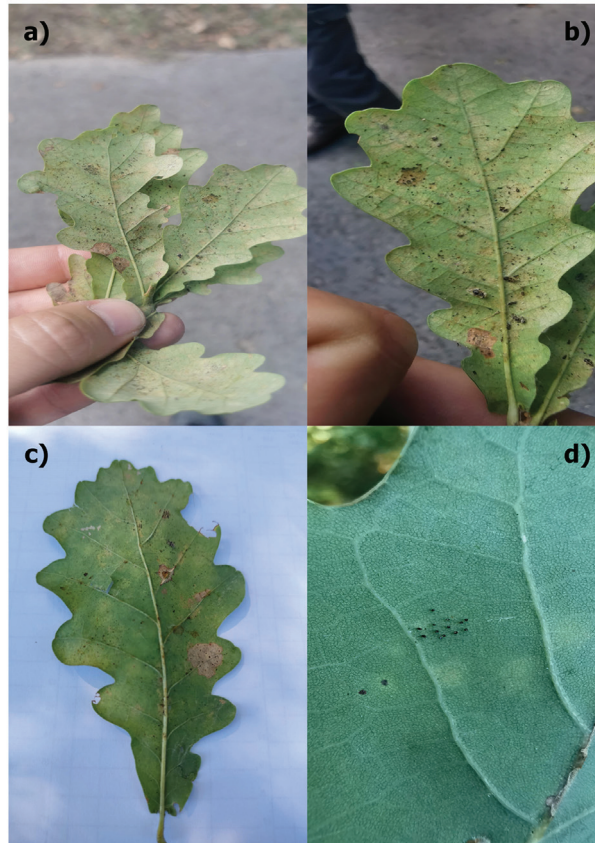


Figure 2. Infested leaves from: (a, b) Site 1; (c, d) Site 2.

This first record in Istrian Peninsula is important as the first record in Sub-Mediterranean oak forests, because other potential host species such as *Q. pubescens* (Willd.) and *Q. ilex* (L.), hosts described by Bernardinelli (2006), surround Motovun forest area (Vukelić et al. 2018). Motovun forest is the only known *Q. robur* forest in Croatia that has not been infected with oak lace bug. The first record on only one oak tree is valuable as we will be able to closely monitor and measure the speed of spread of this highly invasive species. The data on establishment and spread of newly introduced alien species are important for early detection and rapid response on newly introduced alien species (de Groot et al 2020).

Q. pubescens is one of the suitable host plants for OLB (Csoka et al. 2020) and this oak species is dominant in Sub Mediterranean forests in Istria (Vukelić 2018). There are no records of oak lace bug presence on *Q. pubescens* in Croatia so it will be interesting to follow the spread and preference of oak lace bug on *Q. robur* and *Q. pubescens* in Istria as well as in other coastal *Q. pubescens* and *Q. ilex* forests in Croatia. Most of European climate is suitable for OLB (Bernardinelli 2006), so there will be no obstacles for OLB to spread freely in newly invaded regions in coastal Croatia.

OLB has negative influence on photosynthesis, transpiration and stomatal conductance of oak plants (Nikolić et al. 2019). When coupled with other biotic and abiotic stressors, particularly those associated with climate change (Pureswaran et al. 2018), these physiological impacts can negatively affect the health status of oak trees (Nikolić et al. 2019, Pilipovic et al. 2021).

CONCLUSIONS

Detection of oak lace bug (OLB) in the continental region of Croatia, and its subsequent spread to over 200,000 hectares of oak forests, presents a significant concern for

health of oak forests in the region. The first record of this alien invasive pest in the Sub-Mediterranean oak forests on the Istrian Peninsula allows us to track its spread and host preferences in this new environment. Notably, *Q. pubescens*, a dominant oak species in Sub-Mediterranean forests, is a potential host for OLB, which could lead to a further increase in its distribution. Given that the European climate is largely suitable for OLB, its proliferation in newly invaded regions of coastal region of Croatia on suitable host plants seems inevitable. The detrimental impacts of OLB on oak physiology, particularly in conjunction with climate change-related stressors, underscore the importance of early detection and rapid response to mitigate its potential harm to health and productivity of oak trees. Future research will be valuable for monitoring the spread and preference of OLB on different oak species in Istria and other coastal forests in Croatia.

Author Contributions

NZ and MF conceived and designed the research, NZ carried out the field measurements, DM performed laboratory analysis, DM secured the research funding, supervised the research and helped to draft the manuscript, NZ and DM wrote the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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