

The LIFE Programme is the EU's funding instrument for the environment and climate action.

The aim of LIFE SySTEMiC Project (Close-to-nature foreSt SusTainable Management practices under Climate Changes) is to use the "modeling tool" GenBioSivi based on genetic diversity to determine best silviculture practices to protect our forests in times of climate change. The basic idea is simple: the greater the genetic diversity of trees in a forest, the more likely it is that some trees have genetic characteristics that make them more adaptable to climate change, increasing the resilience of the forest system.



Handbook for Sustainable Forest Management

Handbook for Sustainable Forest Management



UNIVERSITÀ DEGLI STUDI
FIRENZE
DAGRI
DIPARTIMENTO DI SCIENZE
AGRICOLE, ALIMENTARI E FORESTALI



CASENTINO
UNIONE DEI COMUNI MONTANI



Zavod za gozdove Slovenije
Slovenia Forest Service



Handbook for Sustainable Forest Management

Deliverable: Handbook for SFM per each species considered



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LIFE SySTEMiC PROJECT DESCRIPTION

The LIFE Programme is the European Union's instrument to finance projects for the conservation of the environment, biodiversity and the fight against climate change.

The aim of the LIFE SySTEMiC Project (Close-to-nature foreSt SusTainable Management practices under Climate Changes) is to use the "modeling tool" based on genetic diversity to identify the best silviculture practices to protect our forests in times of climate change. The basic idea is simple: the greater the genetic diversity of trees in a forest, the more likely it is that some trees have genetic characteristics that make them more adaptable to climate change, thus increasing the resistance and resilience of the forest system.

Based on these premises, the main objectives of the project have been the following:

- To study the relationships between forest management and genetic diversity for eight forest tree species in three European countries (Croatia, Italy, Slovenia) to identify the silvicultural systems that maintain high levels of genetic diversity.
- Development of an innovative Genetic Biodiversity and Silvicultural model (GenBioSilvi) based on the combination of advanced landscape genomics, applied genetics and silvicultural models to support sustainable forest management.
- Disseminate the knowledge of the method across Europe and transfer its use in forestry practice by involving different types of stakeholders.

The Web page of LIFE SySTEMiC project, including detailed protocols:
<https://www.lifesystemic.eu/>



1. INTRODUCTION

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Sustainable, close-to-nature and multifunctional forest management

Sustainable, close-to-nature and multifunctional forest management is planned in a way to preserve forests and all forest functions and ecosystem services, while at the same time guaranteeing profit to forest owners. It can be described by the principles of the "Slovenian Forestry School" as described by Kraigher *et al.* (2019):

- forest management is adapted to site characteristics and natural development of forests;
- active protection of natural populations of forest trees;
- protection and conservation of biodiversity in forests (including genetic diversity);
- supporting the bio-ecological and economic stability of forests by increasing the growing stock;
- tending of all developmental stages and all forest forms for supporting of vital and high-quality forest trees, which could fulfil optimally all functions of forests;
- natural regeneration is supported in all forests;
- if seed or seedlings are used, they should derive from adequate sources/provenances, and only adequate species can be used.

Close to nature forestry uses forest management methods that promote conservation of nature and forests, while deriving tangible and intangible benefits from a forest in a way to preserve it as a natural ecosystem of all its diverse life forms and relations formed therein. It is based on detailed forest management planning, adapted to individual site and stand conditions as well as forest functions, and considering natural processes and structures specific to natural forest ecosystems; it continuously learns from processes in unmanaged forest reserves. Natural processes are altered as little as possible, while still maintaining the financial profitability and social sustainability of forest management. (Forest management by Mimicking nature, 2014).

Close-to-nature forestry mimics natural processes and structures as far as possible. Forest stands should be renewed naturally and should imitate a mixture of tree species and forest stands of natural forests. Forest management can directly influence tree stands in a forest ecosystem. Through natural regeneration of forest stands, trees' adaptability to conditions of specific growing sites and natural dynamics is preserved. Silvicultural systems should be carefully selected in order to promote close-to-nature approaches and mimic natural processes in forest stands.

Forests should be managed in a way to preserve their multifunctional role (ecological, social and productive forest functions). This can be achieved only through maintenance of healthy forests and their biodiversity, protection of their natural fertility and water sources as well as other beneficial functions of forests in the water and carbon cycle, sustainable supply of wood and other products from forest, profit and employment as well as means of recreation and other social benefits related to forests.

Adaptation to individual growing site characteristics is the main direction of close to nature forest development, which has been studied within LIFE SySTEMiC through a variety of sites.

Directed development of forest stands adapted to individual site and stand conditions, and forest functions, demands great flexibility in selection of a proper system (method) of forest management and careful planning of measures.

The most important measures for adapting forest management to climate change focus on adapting the tree composition in forest stands, increasing the resilience of forests through diverse forest stand structures at all levels, especially genetic, through advanced forest regeneration and reforestation measures, and increasing their stability through early maintenance measures (e.g. thinning), the formation of multi-layered and selective forest structures in suitable stands and (not least) the monitoring and conservation of forest biodiversity, starting with genetic diversity (Bajc *et al.*, 2020).

Table 1.1. An overview of different forest management and silvicultural regimes.

Intensity of forest management	Silvicultural system	Description of silvicultural system
Unmanaged	Forest reserve	No intervention, access only permitted for research purposes after authorisation by the regional forestry and nature conservation authority, IUCN protection category I.
Low intensity management	Irregular shelterwood	Rejuvenation in openings and gaps (regeneration cores), in which young trees emerge under the shelter and protection of the mature stand in a mosaic distribution. Later these cores are merged, and each forest stand takes on a new form with a multidimensional structure (adapted after Čater and Diaci, 2020); can be considered IUCN conservation category IV or VI.
	Individual tree selection system	Trees of all dimensions are presented in a small surface area. The regeneration is continuous, and the growth and development of trees are more individualized. The canopy cover is not continuous, but variable, multi-layered and following a stepwise structure. Three main stand positions can be defined in the selection stands: suppressed, waiting individuals in the bottom layer, runners in the middle layer and winners (carriers) in canopy layer (Čater and Diaci, 2020); can be considered IUCN conservation category IV or VI.
Medium intensity management	Replanting after large-scale disturbances	In cases when natural regeneration is not possible or very slow (especially after large-scale disturbances) it is recommended to use artificial regeneration, using seeds or seedlings that are derived from adequate sources/provenances. Only adequate tree species and provenances can be used.

	Uniform shelterwood	The old stand is removed in a series of cuttings (two or three depending on the species to be managed) to promote the establishment of a new even-aged stand under the shelter of the old one. The aim is to protect and shelter the developing regeneration (natural regeneration). The overstory seed-trees are left on site to provide seeds and protect the regenerating understory until the understory no longer needs the protection.
High intensity management	Clear-cutting followed by planting	Regeneration system for even-aged forest. Clear-cutting can also be used when converting monocultures (e.g., spruce) to more diverse stands. The conversion can be done gradually or by clear-cutting followed by planting.
	Outside forest plantations	Planting of tree seedlings outside the forest area.



2. SUSTAINABLE FOREST MANAGEMENT OF *ABIES ALBA* MILL.

2.1 Introduction

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Silver fir is a monoecious wind pollinated and usually allogamous tree species from temperate (including Mediterranean and continental) European mountainous forests (see the distribution map in Figure 2.1.1, EUFORGEN 2009 (www.euforgen.org)).



Figure 2.1.1. Silver fir distribution range (EUFORGEN 2009, www.euforgen.org).

It can grow on a wide range of soil types, except hydromorphic and compressed soils. It is very shade tolerant and can survive under the tree cover for decades. It can grow to a height of 50 to 60 m in pure stands, but usually it is mixed with Norway spruce and/or pine trees at the upper forest range, and with European beech in lower elevations. It may reach its reproductive phase at 20 years, but usually after 60 years of age. The female flowers are mainly on the upper-most branches, while male flowers appear a little lower in the crown. It is considered a weak seed producer since few cones reach maturity due to insects and late frosts. It flowers from April till June, depending on the elevation, and the cones reach maturity in 90 to 120 days. The mature cones are of yellowish - brownish colour, growing upwards on the branches and fall apart when the seeds ripen, so that only the main axis remains. Depending on the site conditions, cones dis-



integrate, and the seeds are dispersed with wind between September and October. Seed collection needs to be well timed, so that the matured cones can be collected (by climbing from standing or from felled trees) before they disintegrate (in Slovenia in mid September). Mast years usually occur periodically (every 4-6 years), but some trees, also depending on the site, can bear cones every year (Kavaliauskas *et al.*, 2020).

The cones can be collected for economical purposes if at least 50% of full seeds are visible on longitudinal cross-cut. One liter of fresh seeds weighs usually around 400 g and the seeds have 8-11% water content. In 1 kg of cones can be 15-30 cones, and in 1 kg of seeds about 14.000-23.000 de-winged seeds. Each cone can bear 260-290 seeds. Seed storage at around 8% water content is possible for 3 to 5 years in hermetically sealed containers at -10 to -15°C. Silver fir embryo is dormant, cold stratification for 3-7 weeks is needed prior to sawing (Kraigher 2024; Regent, 1980; USDA 2008).

Silver fir, especially its saplings, is susceptible to temperature regime, late frosts and prolonged drought. Its regeneration is also highly endangered by browsing, and the changing climate influences its lower resistance to pests and diseases, especially close to the Mediterranean regions. Among pests and diseases, Kavaliauskas *et al.* (2020) specifically mention *Ips typographus* L., *Cinara pectinata* Nördlinger and *Epinotia nigricans* Herrich-Schäffer damage to its bark and buds, and *Armillaria mellea* P. Kumm agg. and *Heterobasidium annosum* Bref. responsible for root and lower trunk rot, leading to damaged silver fir inclination to windthrows.

The silver fir gene pools are well structured latitudinally into a Balkan-South Italian, a Central European-North Italian, an Alpine, a Southern French and a Pyrenean gene pool (GenTree 2020), which is later subdivided into an Eastern and a Western one (Scotti-Saintagne *et al.*, 2021). However, the pattern of distribution of genetic diversity differs depending on the molecular markers used (FORGENIUS 2023; GenTree 2020; Piotti *et al.*, 2017; Teodosiu *et al.*, 2019). Table 2.1.1 reports the list of the sites for *Abies alba* Mill. of the LIFE SySTEMiC project.

Table 2.1.1 List of the sites for *Abies alba* Mill. of the LIFE SySTEMiC project.

Id	Site name	Country	Species	EFT*	Structure	Silvicultural system
06	Faltelli	Italy	<i>A. alba</i>	10.6	Even-aged	Uniform shelterwood
07	Tre Termini	Italy	<i>A. alba</i>	7.3	Uneven-aged	Individual tree selection
16	Gorski kotar, Skrad	Croatia	<i>A. alba</i>	3.2	Uneven-aged	Individual tree selection
26	Smolarjevo	Slovenia	<i>A. alba</i>	3.2	Uneven-aged	Individual tree selection
27	Leskova dolina	Slovenia	<i>A. alba</i>	7.4	Even-aged	Irregular shelterwood
30	La Verna	Italy	<i>A. alba</i>	10.6	Uneven-aged/ Old-growth	Unmanaged

* EFT = European Forest Type: 3.2 Subalpine and mountainous spruce and mountainous mixed spruce-silver fir forest; 7.3 Apennine-Corsican mountainous beech forest; 7.4 Illyrian mountainous beech forest; 10.6 Mediterranean and Anatolian fir forest: 14.1 Plantations of site-native species.

2.2 Forest structure, deadwood and tree-related microhabitats

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Forest structure, deadwood and tree-related microhabitats were inventoried in six sites (Figure 2.2.1).

A permanent observation plot with a representative structure of forest management was selected at each site. The plot had a rectangular shape and each side was 50 m long (area of 2500 m²). Only when the number of trees of the target species (*Abies alba* Mill. with diameter at breast height > 2.5 cm) was < 30 trees another plot was established in the site, until the minimum number of 30 trees was reached. Detailed protocols for the field survey and data elaboration are available on the LIFE SySTEMiC website: <https://www.lifesystemic.eu/>.

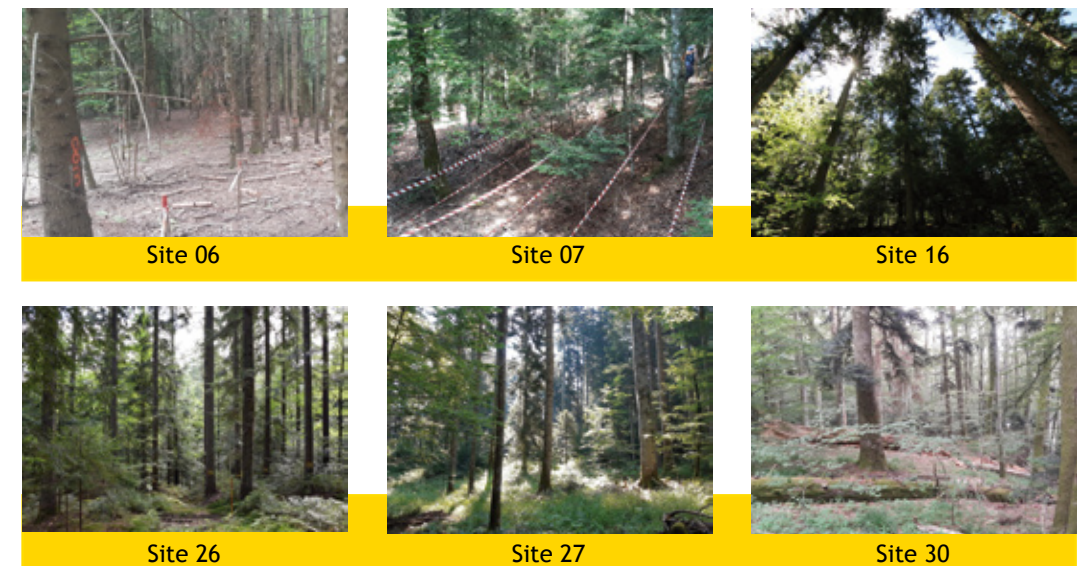


Figure 2.2.1. Sites for *Abies alba* Mill.

Abies alba Mill. was the prevailing tree species (basal area > 75%) in the Sites 16 - Gorski kotar and 27 - Leskova dolina; in the remaining sites *A. alba* was most often mixed with *Fagus sylvatica* L. (Sites 06 - Faltelli, 07 - Tre Termini, 30 - La Verna), *Acer pseudoplatanus* L. (Site 06), *Picea abies* (L.) H.Karst. (Site 26 - Smolarjevo) and other secondary tree species.

The spatial distribution of the trees in the sites is reported in Figure 2.2.2. Figure 2.2.3 shows the stem number - diameter distribution in each site.



Figure 2.2.2. Spatial distribution of the trees in the sites.

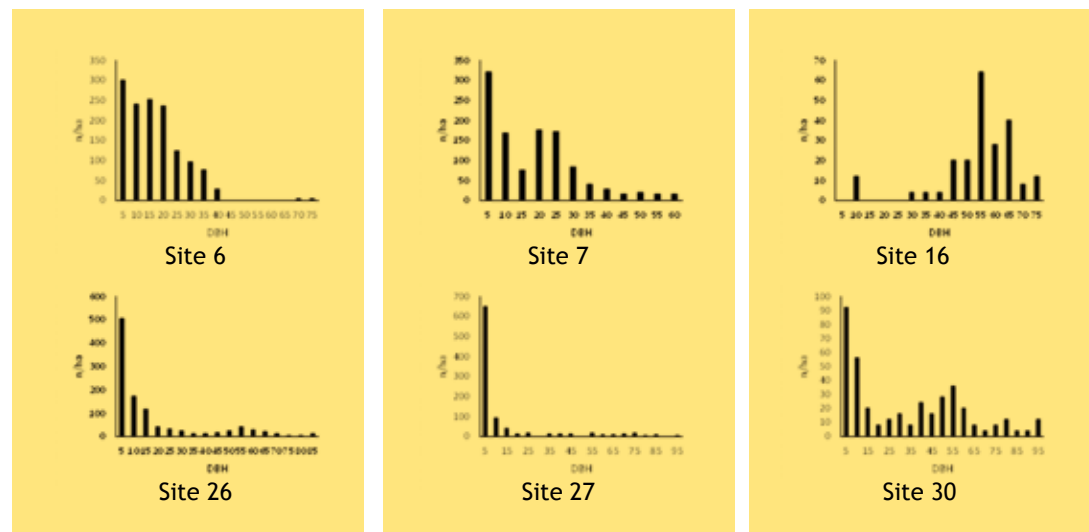


Figure 2.2.3. Stem number - diameter distribution in the sites.

Forest structure variables are reported in Table 2.2.1. In the examined *A. alba* stands, the basal area ranged between 38 m²/ha and 58 m²/ha and the volume varied between 440 m³/ha and 998 m³/ha. In managed even-aged stands (Sites 6 - Faltelli, 27 - Leskova dolina), the basal area was 40 m²/ha and the volume was 496 m³/ha (average values); in managed uneven-aged stands (Sites 7 - Tre Termini,

16 - Gorski kotar, Skrad, 26 - Smolarjevo) the basal area was 52 m²/ha and the volume was 722 m³/ha (average values). The old-growth stand (Site 30 - La Verna) had an uneven-aged structure, the basal area was 56 m²/ha and the volume was 998 m³/ha.

Forest structure diversity, represented by the trees dimensional diversity (standard deviation and coefficient of variation of DBH and heights), was high in the old-growth stand (SDDBH = 26.9%, SDH = 14.4%) followed by uneven-aged (SDDBH = 15.7%, SDH = 9.1%) and even-aged (SDDBH = 14.8%, SDH = 8.6%) stands.

Table 2.2.1. Forest structure variables in the sites. N=number of stems; BA=basal area; V=volume; QMD=quadratic mean diameter (diameter of the mean basal area tree); MH= mean height (height of the mean basal area tree); DH= dominant height (mean height of the 100 trees per hectare with the largest diameters in case of even-aged stand, and mean height of the three tallest trees per hectare in case of uneven-aged stand); SDDBH= standard deviation of DBH; SDH= standard deviation of total tree heights; CVDBH= coefficient of variation of DBH; CVH= coefficient of variation of total tree heights.

Id	N	BA	V	QMD	MH	DH	SDDBH	SDH	CVDBH	CVH
	n/ha	m ² /ha	m ³ /ha	cm	m	m	cm	m	%	%
06	1360	41.4	440.3	19.7	19.2	26.0	10.3	6.6	61.8	41.4
07	1132	44.6	504.7	22.4	19.3	27.2	13.1	8.5	72.3	59.2
16	216	53.5	882.6	56.2	33.5	39.5	14.1	7.2	26.0	22.4
26	1072	57.6	779.4	26.2	24.0	45.0	19.8	11.6	115.2	88.6
27	920	38.2	550.6	23.0	21.6	37.0	19.3	10.5	154.3	118.1
30	388	56.0	997.8	42.9	30.6	45.8	26.9	14.4	80.1	66.5

For each site, the amount of deadwood is reported in Table 2.2.2. In the examined stands, the total volume of deadwood ranged between 14 m³/ha and 426 m³/ha. In managed even-aged stands (Sites 06 - Faltelli, 27 - Leskova dolina), the total volume of deadwood was 24 m³/ha (average value), which was mostly represented by stumps (59%) and snags (22%); in managed uneven-aged stands (Sites 07 - Tre Termini, 16 - Gorski kotar, Skrad, 26 - Smolarjevo) the total amount of deadwood was 48 m³/ha (average value), that was mostly represented by snags (43%) and stumps (32%). The old-growth stand (Site 30 - La Verna) had the largest amount of deadwood (426 m³/ha), represented by snags (39%), downed dead trees (31%) and other lying deadwood pieces (29%).

Table 2.2.2. Volume of deadwood in the sites.

Id	Standing dead trees (including snags)	Downed dead trees	Other lying dead wood pieces	Stumps	Total
	m ³ /ha	m ³ /ha	m ³ /ha	m ³ /ha	m ³ /ha
06	10.0	0.0	3.8	2.3	16.1
07	17.2	0.0	0.4	14.0	31.6
16	44.0	23.7	8.7	23.1	99.5
26	1.2	1.5	2.0	9.1	13.8
27	0.6	0.4	4.9	25.9	31.8
30	168.0	129.8	122.5	5.7	426.0

The frequency of the tree-related microhabitats at each site, represented as the percentage of forms of microhabitats, is shown in Figure 2.2.4. Cavities and epiphytes were the most common forms of microhabitats at all sites, except Site 27 - Leskova dolina where the most frequent forms were deformation / growth forms and injuries and wounds.

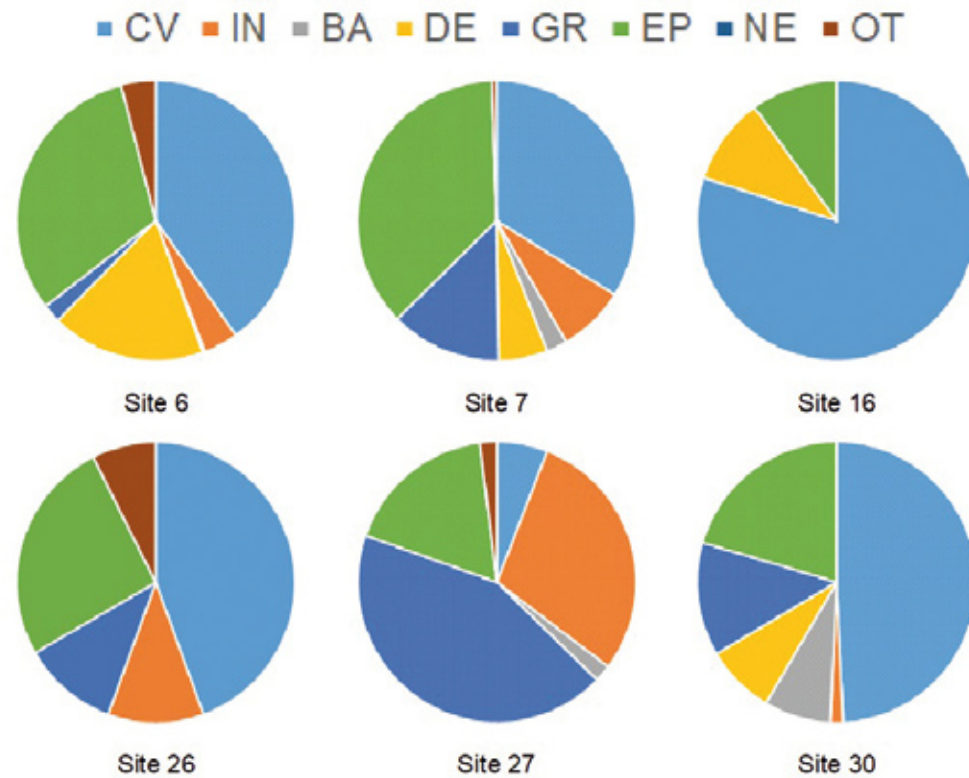


Figure 2.2.4. Tree-related microhabitats in the sites (percentage of forms of microhabitats) (CV = Cavities; IN = Injuries and wounds; BA = Bark; DE = Deadwood; GR = Deformation / growth form; EP = Epiphytes; NE = Nests; OT = Other).



Figure 2.2.5. Site 30 - La Verna old-growth forest.



Figure 2.2.6. Site 30 - La Verna old-growth forest.

2.3 Landscape genomics

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Tree populations can follow two main strategies to cope with major changes in climatic conditions: migration and adaptation (Aitken *et al.*, 2008). Migration rates are likely to be outpaced by the current rate of climate change (Davis *et al.*, 2001) and could be hindered by natural barriers and human-induced habitat transformations (Hoegh-Guldberg *et al.*, 2008). On the other hand, the genetic diversity of populations could be key to mitigating the effects of climate change (Mosca *et al.*, 2012) and enable adaptation through the selection of potentially beneficial alleles present in the populations (Barret *et al.*, 2008). Genetic variation is crucial for the fitness and survival of individuals within a population, and it is directly correlated with the species' ability to adapt to different environments (Balkenhol *et al.*, 2016). Within each population, genetic changes, plasticity and evolvability result from the combination of random and selectively oriented processes, that can be influenced by forestry practices (Lefèvre *et al.*, 2014). Understanding the dynamics and mechanisms of local adaptation in natural populations provides basis for predicting responses to environmental changes, including those associated with global climate change. Local adaptation is expected to alter the allele frequencies of genes that influence fitness in specific environments (Lefèvre *et al.*, 2014). Therefore, understanding the relationship between genomes and adaptive phenotypic characteristics and the extent to which they are influenced by the environment may be essential for predicting the fate of tree species coping with climate change (Babst *et al.*, 2019; Alberto *et al.*, 2013).

Abies alba Mill., is one of the most important conifer species in the mountainous regions of Mediterranean Europe. As climate change progresses, silver fir has attracted renewed interest due to its surprising drought tolerance observed in Central and Southern Europe (Carrer *et al.*, 2010; Tinner *et al.*, 2013; Vitali *et al.*, 2017; Vitasse *et al.*, 2019). Although it was generally agreed that this conifer is quite resistant to drought (Bouriaud *et al.*, 2009; Elling *et al.*, 2009), water stress has been linked to growth decline, crown defoliation, and uncoupled patterns of seed dispersal and regeneration in recent decades (González de Andrés *et al.*, 2014, 2022). Predictions point to more frequent and intense droughts in the Mediterranean area, concurrent to global warming, which will further challenge the species' ability to cope with the new environmental conditions (Ozturk *et al.*, 2014). In this context, landscape genomics has emerged as a flexible analytical framework for understanding the interactions between environmental heterogeneity and adaptive genetic variation in natural populations (Balkenhol *et al.*, 2016). We used Landscape Genomics approaches to analyse the neutral and adaptive component of genetic diversity to highlight possible patterns of local adaptation in the populations. Therefore, we correlated adaptive molecular markers, specifically Single Nucleotide Polymorphism (SNPs) present in candidate genes associated with abiotic stress, with bioclimatic indicators. Nuclear microsatellite markers (nSSR) were analysed as a measure of neutral genetic variation and structure of the studied populations. Single Nucleotide Polymorphism (SNPs) genotyped using a target re-sequencing approach of candidate genomic regions, were analysed as a measure of adaptive genetic variation of the studied population (Figure 2.3.1). As a result of *A. alba* target re-sequencing about 1500 SNPs were observed in 24 genome regions relevant for response to one or more abiotic stresses (results reported in deliverable Action B1: SNP road-map of each study site). In addition, we investigated the distribution of these SNPs at the population level, and we have designed road-maps for each target species in order to observe their spatial distribution among all the analysed populations (Figure 2.3.1).

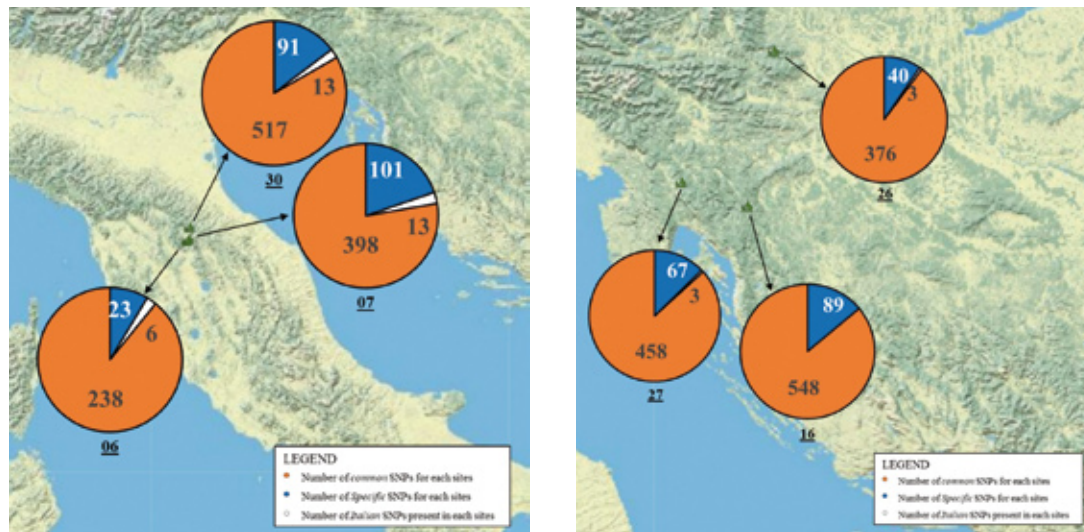


Figure 2.3.1. The road-map of *A. alba* SNPs distribution in LIFE SySTEMiC sites of Italy (A) and Croatia-Slovenia (B) is depicted in the figure. The figure shows the number of certain specific subsets of SNPs for each site. The data types are sorted by colour. The colours represent the following SNPs subset: the set of SNPs present in that site which are common to at least two sites of interest (orange); the number of unique SNPs specific to that site (white) and the number of SNPs present in that site, which are specific to the country of origin (blue). Below each graph, the identifying number of the corresponding site is displayed (in bold and underlined).

A higher number of site-specific and region-specific SNPs can be observed for sites located in Italy (Figure 2.3.1). This prevalence of SNPs could be interpreted as a sign of adaptation to a Mediterranean bio-climatic regime that characterizes the Italian peninsula and sets it apart from the more continental climate found in Slovenia and Croatia. As stated in bibliography, analysis for detecting the signature of local adaptation in natural populations requires many analytic steps (Blanquart *et al.*, 2013). For each site, twelve bio-climatic indicators were selected from the existing literature (Flint *et al.*, 2013; Gugger *et al.*, 2016, 2021; Pluess *et al.*, 2016). To identify local adaptation patterns of the target species, we conducted the Genome Environment Associations (GEA) analyses on two levels: global and site-specific analysis. The global analysis allowed us to identify possible patterns of adaptation to the bioclimatic conditions that characterize the range of silver fir. The results of the analysis showed the existence of four different clusters present in Italy, Croatia and Slovenia. Additionally, an even more interesting finding is the presence of association between 78 allelic variants and the mean values of the 12 bioclimatic indicators considered for these analyses (as reported in Deliverable Action B1: Production of maps of spatial distribution of genetic diversity and of correlation between allele distribution and environmental variation). The presence of these associations could be interpreted as the basal adaptation genotype of silver fir spread in the Central European range. Of particular interest was finding some site-specific associated allelic variants. The presence of these allelic variants could be correlated with the local rather than regional pattern of adaptation. In the environmental association analysis (EAA) it is important to account for neutral genetic structure (Rellstab *et al.*, 2015), as neutral genetic structure can produce patterns like those expected under non-neutral processes (Excoffier and Ray, 2008; Excoffier *et al.*, 2009; Sillanpää 2011). Furthermore, the genetic structure of populations was analysed using STRUCTURE (Pritchard *et al.*, 2000) and spatial GENELAND software (Guillot 2008).

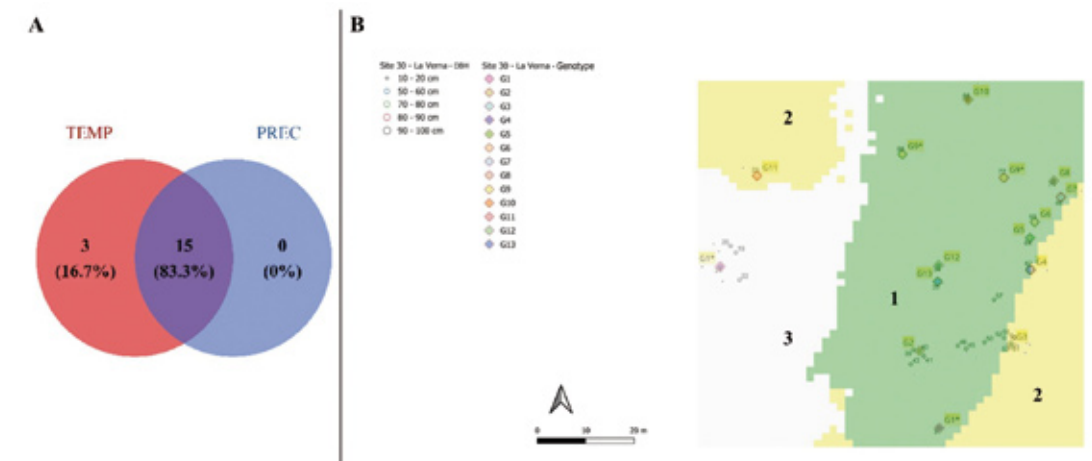


Figure 2.3.2. LFMM analysis results and genotype distribution map of Site 30 - La Verna. (A) Venn diagram showed the overlapping between SNP associated with temperature-related and precipitation-related bioclimatic indicators, as a result of LFMM analysis. (B) Spatial distribution of genotype and spatial organization in 3 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter is characterized by coloured circles according to the genotype observed. Identical colours mean identical genotypes.

In general, we found a moderate to high number of specific allelic variants in unmanaged sites (i.e. Site 30 - La Verna; Figure 2.3.2). The presence of a high number of SNPs associated with adaptation to bioclimatic indicators at these sites could be correlated with the neutral genetic structure observed for these sites (Aravanopoulos, 2018; Paffetti *et al.*, 2012; Stiers *et al.*, 2018). Some studies have observed that these sites are characterized by a complex neutral genetic structure, characterized by a high number of family clusters. This great variability may allow a higher probability for the appearance of new allelic variants that could enrich the adaptive potential of this species for current and future climate change. A similar situation was observed in stands managed with reduced-impact management types: individual tree selection. Once again, the number of allelic variants specific to each site is high. When analysing the distribution pattern of genetic diversity distribution based on nuclear microsatellite (nSSR) data, we observed that silver fir stands managed according to individual tree selection system have a complex and heterogeneous spatial genetic structure. This can be attributed to the non-random mating between closely related individuals. An interesting finding is the number of allelic variants associated with bioclimatic indicators that characterize the local environment found at the Site 07 - Tre Termini (Figure 2.3.3).

Due to its extraordinary ability to disseminate and root, silver fir renews easily, which allows management close to nature; the forest shows natural regeneration without any human planting intervention. Problems with renewal occur only in overused stands (usually in forests that do not have a controlled management system). From the results obtained for each studied stand, it was possible to observe a simplified spatial genetic structure in unmanaged/old-growth fire forests in respect to those sites. This can be due to less natural regeneration than managed sites. Site 07 - Tre Termini and Site 26 - Smolarjevo (Figure 2.3.4), managed according to individual tree selection, reported the most complex spatial genetic structure among the managed sites (respectively, 6 and 5 genetic clusters) as well as, the highest number of SNPs associated with bioclimatic indicators. The complexity reported for those sites implies a dynamic and adaptable ecosystem capable of responding to environmental changes by generating new genetic variability through recombination and gene flow between subpopulations. In addition, we observed a high number of SNPs correlated with bioclimatic indicators across sites. The presence of these allelic variants associated with bioclimatic indicators that best characterize the local environment is important for adaptation.

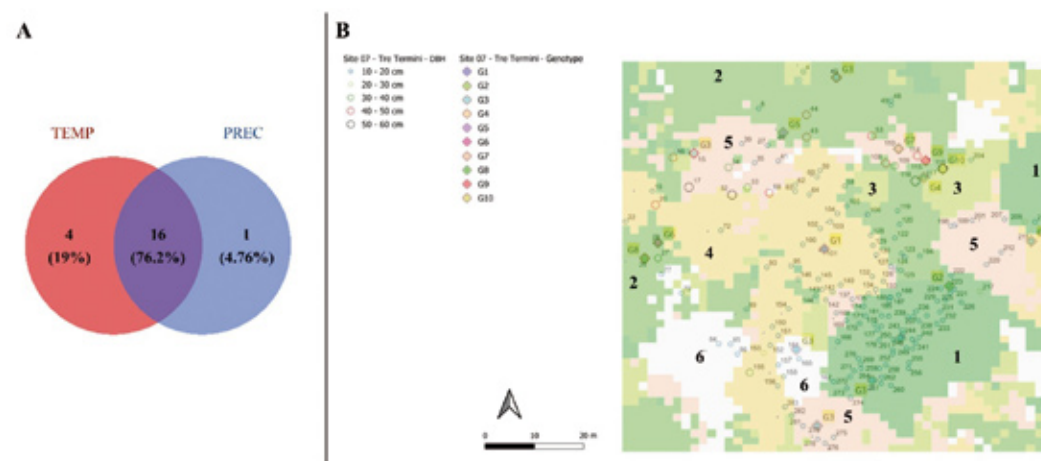


Figure 2.3.3. LFMM analysis results and genotype distribution map of Site 07 - Tre Termini. (A) Venn diagram showed the overlapping between SNP associated with temperature-related and precipitation-related bioclimatic indicators, as a result of LFMM analysis. (B) Spatial distribution of genotype and spatial organization in 6 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter is characterized by coloured circles according to the genotype observed. Identical colours mean identical genotypes.

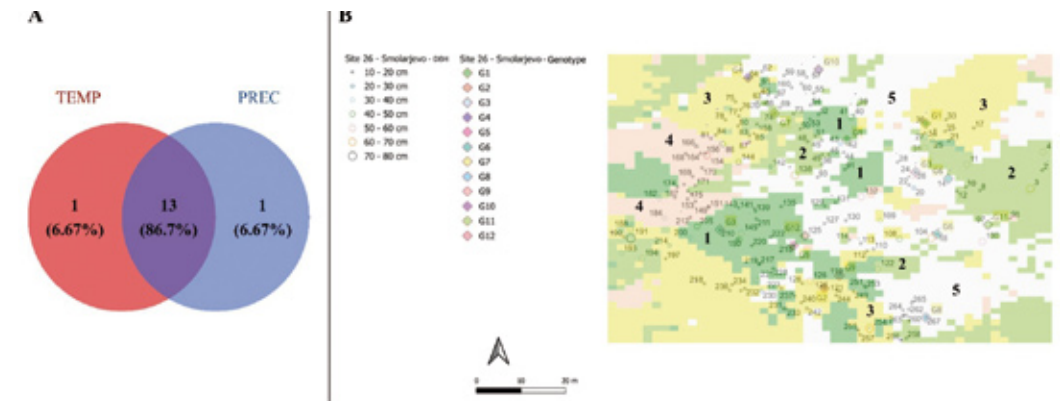


Figure 2.3.4. LFMM analysis results and genotype distribution map of Site 26 - Smolarjevo. (A) Venn diagram showed the overlapping between SNP associated with temperature-related and precipitation-related bioclimatic indicators, as a result of LFMM analysis. (B) Spatial distribution of genotype and spatial organization in 5 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter is characterized by coloured circles according to the genotype observed. Identical colours mean identical genotypes.

The creation of gaps in forest cover and the complexity of structure characterizing these stands could be linked to a higher probability of gene recombination between genotypes belonging to different family clusters. The potential for reduced-impact silvicultural management to conserve adaptive genetic diversity appears promising. An intriguing observation is the presence of a high number of associated SNPs and a complex spatial genetic structure (4 clusters) in Site 06 - Faltelli.

Less impactful management types, such as individual tree selection, appear to report population with a high number of allelic variants associated with response to bioclimatic indicators. Similar results have been observed in unmanaged stands and old-growth forests. The results reported in this study could play an instrumental role in silvicultural management planning, where knowledge of genetic variability from an adaptive perspective could help decision-making processes. This is important to preserve the current Forest Genetics Resources (FGR), but also to enrich the existing stand with potentially favourable genotypes.

2.4 Soil biodiversity

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Biodiversity at all levels (i.e. genetic, species, ecosystem) supports sustainable forest dynamics, including diverse functions and biology of forest tree species and their genetic diversity. In European temperate forests the majority of stand forming tree species are symbiotic with ectomycorrhizal fungi (Kraigher *et al.*, 2013). With extramatrical mycelium, which is formed by the emanating hyphae and/or rhizomorphs (i.e. bundles of hyphae) ectomycorrhizal fungi grow in the surrounding substrate and take up nutrients and water from the compartments that are inaccessible for tree roots (Finlay, 2008). The functional compatibility in symbiosis depends on the species and provenance/population of forest trees and on the species and strain of the symbiotic fungi (Gianinazzi-Pearson, 1984; Hazard *et al.*, 2017). Therefore, the identification of the mycorrhizal fungi is of utmost importance for understanding the functioning of forest ecosystems.

Diversity of silver fir ectomycorrhizae was investigated at Site 26 - Smolarjevo from March 2016 to October 2017 in four-week intervals by combination of morpho-anatomical approach with Sanger sequencing (Unuk Nahberger, 2020). Five trees at 5-7 m distance from each other were included in the study and 500 root tips were examined in total per tree in each sampling campaign (sum of vital and non-vital included in this number). All-over, 42 taxa were reported. The ten most abundant taxa were *Thelephora wakefieldiae*, *Russula ochroleuca*, *Elaphomyces granulatus*, *Xerocomellus pruina-tus*, *Lactarius subdulcis*, *Neoboletus erythropus*, *Cenococcum geophilum*, *Tomentella stiposa*, *Rus-sula badia* and *Tylospora fibrillosa*. High relative abundances of *Thelephora*, *Tylospora* and *Lactarius* at Site 26 were explained by the relatively high soil nitrogen content (0.62%) at this location which could also result in the absence or low relative abundance of nitrogen-sensitive taxa, such as *Corti-narius*, *Tricholoma* and *Piloderma*.

Sampling month was a significant determiner of the ectomycorrhizal vitality ($p=0.0419$), species richness ($p=0.0012$), evenness ($p=0.0018$) and dominance ($p=0.0102$) in Site 26. In 2016, the highest proportion ($p<0.05$) of vital ectomycorrhizae was observed for July and August and the lowest in March and May. In 2017, the highest vitality was observed in May and the lowest in June. The highest ectomycorrhizal vitality followed or coincided with the time of the budburst in *Abies alba* Mill. Sum-mer-time was generally marked by the decreased species richness, evenness, and lower abundance of the most abundant and rare taxa. These changes were explained by the dynamics of fine root growth, where two peaks of fine root growth in spring and autumn are interrupted by the period of the relative inactivity of fine roots in summer (Unuk Nahberger, 2020).

2.5 Browsing

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In the last century, the density and spatial distribution of large herbivores, especially roe deer (*Capreolus capreolus*, (Linnaeus, 1758)) and red deer (*Cervus elaphus* (Linnaeus, 1758)) has increased significantly in most of European countries, including Slovenia (Hafner *et al.*, 2020). Ungulate browsing is known to have significant impacts on forest ecosystems. Ungulate game selectively target certain tree species or individuals, giving an advantage to other species that are less preferred by browsing animals. Therefore, browsing can strongly impact the structure, composition, growth and succession of forest. That may in the long run lead to species diversity reduction and threaten the resilience of the forest to future disturbances.

As part of the B3 activity in LIFE SySTEMiC project, we aimed to investigate whether the impact of ungulate browsing in areas with high wildlife density, and consequently high browsing pressure, can also be detected in the genetic diversity of natural regeneration. This research involved comprehensive experimental planning, the establishment of research plots (both fenced and unfenced) for four target species - Silver fir (*Abies alba* Mill.), European beech (*Fagus sylvatica* L.), Stone pine (*Pinus pinea* L.) and Pedunculate oak (*Quercus robur* L.) - in Slovenia and Italy (Figure 2.5.1), and extensive inventories of natural regeneration of forest tree species (Figure 2.5.2). The importance of the impact of ungulate browsing and the description of our study is described in this chapter, because of the 4 species, a more extensive study was carried out on this species, which also included genetic analyses.



Figure 2.5.1. Wildlife browsing experiment sites.

Plot establishment was carried out following a protocol 'Ungulate browsing experiment protocol' described in LIFE SySTEMiC technical manual available on the project website (www.lifesystemic.eu). Four pairs of plots - one fenced and one unfenced - representing four replications were established. Each plot had a size of 12.5 x 12.5 m. We marked plots' corners with wooden stakes and coloured them with permanent paint. Plots, chosen to be protected from ungulates, were fenced around the stakes. By comparing the species composition of natural regeneration and adult trees, we sought to quantify the intensity of wildlife browsing pressure. At one of the sites, in Leskova dolina, Slovenia, we conducted a second inventory of the silver fir natural regeneration two years after the initial survey. This follow-up aimed to determine whether changes in the natural regeneration abundance could be detected over such a short period. Inventory was followed by sampling: we sampled both, the silver fir saplings and the adult trees. Our aim was to compare indicators of genetic diversity, based on nSSR markers, between the saplings in the unfenced and fenced plots, and in comparison to the adult (potential parent) trees. In particular, we were interested in any potential reduction in genetic diversity of saplings in unfenced plots, exposed to browsing pressure, compared to fenced plots and mature trees, two years after fencing.



Figure 2.5.2. Sapling height measurement (left), browsing damage assessment (middle) and inventory (right).

In silver fir sites with high densities of ungulate animals, the impact of browsing on natural regeneration is well-known among forest managers. In our study, this primarily concerns the experimental sites Leskova dolina and Faltelli, where we observed various effects of browsing:

- **Low abundance of silver fir saplings in higher height classes**, particularly in the height class above 150 cm, which was sometimes completely absent in our research sites. This threshold height is of crucial importance, as it marks the point where the impact of browsing ungulates on forest composition becomes negligible. This height class thus provides a foundation for the design of future forest stands (Hafner *et al.*, 2020). The absence of saplings in this class suggests that browsing pressure might be hindering the successful establishment of certain species. Ideally, natural regeneration would be represented across all height classes. The presence of saplings in the lowest height class indicates sufficient seed production and initial establishment. However, successful growth into higher height classes signifies the saplings' ability to withstand environmental disturbances and remain competitive.
- **Lower proportion of silver fir in natural regeneration compared to the adult population.** This disparity is most evident in Leskova dolina and can be at least partly attributed to ungulate browsing. Preferred species lose their competitiveness primarily due to browsing of terminal buds. Strong ungulate browsing results in a decrease in the height of these browsed species in the regeneration layer, significantly impacting their competitive ability (Horsley *et al.*, 2003; Tremblay *et al.*, 2007). Browsing heavily impacts palatable species, leading to a dominance of less palatable species such as Norway spruce (*Picea abies* (L.) H. Karst.). This shift can reduce overall biodiversity and affect forest regeneration processes (D'Aprile *et al.*, 2020).
- **High damage to saplings.** The highest proportion of damaged saplings was observed in silver fir, with the most browsing damage occurring in the 11-50 cm and 51-150 cm height classes. Among the deciduous trees, sycamore maple (*Acer pseudoplatanus* L.), rowan (*Sorbus aucuparia* L.), and holm oak (*Quercus ilex* L.) were the most palatable species on our research plots, while European beech was mostly undamaged by browsing. Studies from central and southeastern Europe (Shulze *et al.*, 2014) illustrate that deer, by preferentially consuming certain species, contribute to the homogenization of the forest understory, potentially impacting long-term forest structure and biodiversity.

Despite the noticeable effects of ungulate browsing on natural regeneration structure and composition, no significant genetic effects were detected. Genetic diversity did not differ significantly between adult silver fir trees and their regeneration, whether in fenced or unfenced plots.

The impact of ungulate browsing on different species and growth stages of forest trees varies significantly. Young forests, particularly during early growth stages, often face heightened browsing pressure, severely affecting the survival and growth rates of tree saplings. Species such as oak and beech, more resistant to browsing, might withstand this pressure better than fir and pine, which are more susceptible. Differential browsing impacts can lead to changes in forest composition over time, favoring more resistant species and potentially altering the forest ecosystem (Hafner *et al.*, 2020).

Effective forest management strategies must consider these dynamics to support diverse and resilient forest ecosystems. Forest managers might implement controlled browsing, protective measures like fencing, and promote a mixed species composition that includes more browsing-resistant trees (Hafner *et al.*, 2020). Alternative management strategies for ungulate populations may provide “windows of opportunity” for forest regeneration in time and/or space (Didion *et al.*, 2009). Additionally, integrating wildlife management to regulate herbivore populations can minimize negative impacts, ensuring that forest ecosystems remain resilient and productive. Such strategies are crucial for balancing conservation goals with the ecological realities of herbivorous wildlife interactions (Hafner *et al.*, 2020).

2.6 GenBioSilvi model

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The development of the GenBioSilvi model is based on the forest ecosystem biodiversity conservation and on the sustainable forest management in response to abiotic stress. Therefore, the GenBioSilvi model focused on the definition of genetic diversity indicators and other forest ecosystem biodiversity indicators across different European forest types (EFT) to identify optimal silvicultural practices to conserve/enhance the genetic diversity that confers the ability to respond to climate change.

Measuring genetic diversity is crucial for understanding the state of resilience found forest ecosystems. When analysing the distribution pattern of genetic diversity distribution using nuclear microsatellite (nSSR) data, we found that fir stands managed according to individual tree selection system have a complex and heterogeneous spatial genetic structure. This last characteristic increases the possibility of sexual recombination among the individuals in the above ground stands and thus their ability to respond to climate changes.

Based on the results obtained for each stand analysed (see the deliverable Action B2 GenBioSilvi model), it was possible to observe a simplified spatial genetic structure in unmanaged/old-growth forests. This can be due to less natural regeneration presence than in managed site. Site 07 - Tre Termini and Site 26 - Smolarjevo, managed according to individual tree selection, reported the most complex spatial genetic structure among the managed sites (respectively, 6 and 5 genetic clusters). The complexity reported for Site 07 - Tre Termini implies a dynamic and adaptable ecosystem capable of responding to environmental changes by generating new genetic variability through recombination and gene flow between subpopulations. In addition, we observed a high number of SNPs correlated with current environmental conditions across sites. We found that Site 16 - Gorski Kotar, Skrad (individual tree selection), and Site 07 - Tre Termini (individual tree selection) presented a higher number of SNPs correlated with bioclimatic indicators.

Based on our results, we assumed that better conditions are found in individual tree selection systems.

To describe forest diversity structure, we observed dendrometric data. A multi-layered uneven-aged population supports the presence of natural regeneration because it shows gaps in canopy cover that create ideal conditions for the establishment of natural regeneration. Furthermore, a multi-layered forest increases the probability of sporadic species occurrence. In addition, multi-layered forest allows high pollen dispersal, promoting genetic diversity. Therefore, based on the parameters used to define forest structure, we observed that sites characterized by multilayered uneven-aged forest structure also exhibit complex spatial genetic structure and high genetic diversity. Site 16 - Gorski Kotar, Skrad, managed by principles of individual tree selection, showed a bell-shaped mono-stratified forest structure that implies the absence of natural regeneration. Instead, Site 30 - La Verna, unmanaged forest, showed a j-shaped bi-stratified forest structure.

The presence of deadwood can promote germination and seedling development of tree species, acting as nursery sites and thus contributing to forest renovation. Regarding the amount of deadwood, the best situation is observed in unmanaged sites that showed a higher volume of deadwood.

Deadwood provides an important microhabitat for the development and preservation of species that are important for the forest ecosystem. In addition to deadwood, we observed other indicators related to saproxylic microhabitats. The largest number of microhabitats was detected at sites where there are century-old individuals with stem deformations and cavities. The frequency of epixylic microhabitats is lower than saproxylic in all observed populations. Epixylic are used as indicators to assess ecosystem conditions. The presence of these microhabitats is an important source of biodiversity. Based on the obtained results, management types that allow for complex forest structures characteristic of uneven-aged and multilayered stands increase the probability of observing different microhabitat forms.

All analyzed sites are mixed fir stands. In effect, fir is a sciaphilous species that rarely forms pure forests. Instead, it is an important component of mixed forests in the montane and subalpine planes, e.g., it can be associated with beech (*Fagus sylvatica* L.), which has similar climatic and soil requirements, while at subalpine altitudes it can be associated with larch (*Larix decidua* Mill.) and spruce (*Picea abies* (L.) H.Karst.).

When analysing the results, we found that the condition that maintains and/or increases diversity is the managed site with individual tree selection. This type of management is less impactful moreover it mimics conditions normally found in old-growth forests and unmanaged forests. The removal of a few individuals based on phenotype and position leads to in the formation of gaps in the tree cover that promote the growth of natural regeneration. Compared to unmanaged fir stands, in which there is no natural regeneration or it is present only in marginal areas, stands managed according to individual tree selection exhibit gaps in forest cover and provide optimal conditions for the establishment of natural regeneration.

This type of management increases the complexity of the forest structure presenting a multi-layered pattern, a typical characteristic of an uneven-aged forest. This allows better pollen dispersal -high gene flow- since fewer barriers are present. This promotes genetic diversity and increases spatial genetic complexity, leading to a greater occurrence probability of new allelic variants that are essential in responding to climate change. In addition, a key aspect of forest stand resilience is the presence of high biodiversity. Through our results, we have observed that stands that exhibit higher genetic diversity also exhibit higher biodiversity at the ecosystem and species levels.

Based on the results obtained during the analyses and the forest ecosystem indicators calculated, it is possible to simulate the genetic diversity status of the stand and guide users toward sustainable management to conserve and/or increase the genetic diversity present.

Taking into account the results obtained from the analysis of all biodiversity indicators, we have developed a model that describe the current status of genetic resources within the site. This model was developed to help forest users to check the current status of biodiversity in the stands and to create guidelines for sustainable management. To develop a user-friendly suitable model, we observed that it is possible to identify a set of key indicators that are more representative. The indicators on which the model is based can indirectly describe the genetic diversity status of the stand, as shown by the results obtained. In addition, it is also possible to identify some indicators that are more representative of biodiversity in terms of deadwood, microhabitat, and species diversity. For this reason, we decided to exclude in the form developed for users the data collection on genetic diversity and soil diversity that are difficult to observe. In this context, we have developed a model that includes the observation of some key indicators that describe the current status of the analysed stand. The selected indicators are listed in Table 2.6.1.

Table 2.6.1. Description of selected indicator useful for users to describe the status of the stand.

Categories	Indicators	Description
Forest structure	DBH standard deviation	Variability in tree DBH within the stand
	Percentage of trees in regeneration layer	Presence of the target species natural regeneration
	DBH class distribution curve	Complexity of horizontal and vertical forest structure
	No. of population strata	
Deadwood	Standing deadwood presence	Description the presence of deadwood functioning as microhabitat
	Coarse woody debris	
Species diversity	Species richness	Number of all species present in the stand regarding both the presence of adults' individuals and regeneration
	Percentage of non-target individuals regeneration	
	Percentage of individuals with cavities	Presence of key microhabitat form for biodiversity
	Percentage of individuals with Injuries and wounds	
	Percentage of individuals with Deformation	



The model involved the production of a form that requires the compilation of certain parameters that can be easily observed in the field by users. Based on our results, we have chosen value thresholds for each selected indicator. The annotation of the actual real data for each indicator will produce a score based on the selected thresholds. The final score is associated with the identification of the current status of the stand analysed. For each final score, indications are provided regarding possible actions to be taken to implement sustainable management of the stand (Figure 2.6.1).

Below is the example of the form we produced for compilation (Figure 2.6.2). Instead, Figure 2.6.3 shows the compiled form based on the actual data obtained from the Site 07 - Tre Termini.

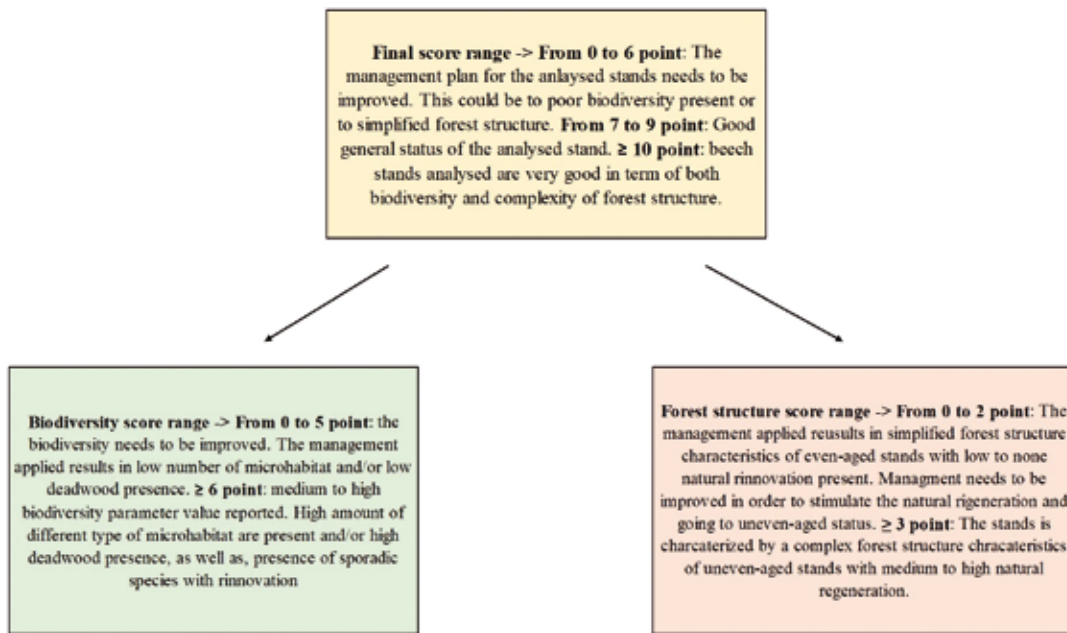


Figure 2.6.1. Guideline for forest management based on the final score obtained after forest assessment forest form compilation.



Forest stand evaluation form			
Forest management	Real Data	Indicator specific score	Implication for Management
Forests should be managed in a way to preserve their multifunctional role (ecological, social and productive forest functions). This can be achieved only through maintenance of healthy forests and their biodiversity, protection of its natural fertility and water sources as well as other beneficial functions of forests in the water and carbon cycle, sustainable supply of wood and other products from forest, profit and employment.			
Forest structure indicators	Real Data	Indicator specific score	Implication for Horizontal and Vertical Forest structure
Forest structure is both a product and driver of ecosystem processes and biological diversity. Changes in forest structure as a result of management for timber production have undesirable consequences for other components of forest ecosystems	Structural indicator		
	DBH Standard deviation	<input checked="" type="checkbox"/> Score 1 (DBH SD > 5) <input type="checkbox"/> Score 2 (7 < DBH SD <= 11) <input type="checkbox"/> Score 3 (DBH SD > 15)	Results from associated parameters
	Refers to % of trees in regeneration layer (including DBH less than 10 cm)	<input checked="" type="checkbox"/> Score 1 (%Rin < 15) <input type="checkbox"/> Score 2 (15 < %Rin < 30) <input type="checkbox"/> Score 3 (30 < %Rin < 50) <input type="checkbox"/> Score 4 (%Rin > 50)	<input type="checkbox"/> Score 1 (Simple forest structure without rinnovation) - From 4 to 6 <input type="checkbox"/> Score 2 (Simplified forest structure with rinnovation or Complex forest structure without rinnovation) - From 7 to 8 <input type="checkbox"/> Score 3 (Complex forest structure with rinnovation) - From 9 to 11 <input type="checkbox"/> Score 4 (Complex forest structure with rinnovation present in the total area) - From 12 to 13
	Curve of DBH class distribution	<input type="checkbox"/> Score 1 (Bell-shaped) <input type="checkbox"/> Score 2 (Skirt-shaped) <input type="checkbox"/> Score 3 (J-shaped)	
N° of strata (stratification of population)	<input type="checkbox"/> Score 1 (Mono-stratified) <input type="checkbox"/> Score 2 (Bi-stratified) <input type="checkbox"/> Score 3 (Tri or Multi-stratified)		
Productivity indicators	Real data	Indicator specific score	Implication for population biodiversity
Forest site productivity is the production that can be realized at a certain site with a given genotype and a specified management regime. Site productivity depends both on natural factors inherent to the site and on management related factors.	Growing stock (m³/ha)		In managed stands, the minimum growing stock should be around 200-300 m³/ha. This might be applicable for shade tolerant species
Biodiversity indicators	Real data	Indicator specific score	Implication for stand biodiversity
Deadwood indicators	Standing deadwood (including snag) presence	<input type="checkbox"/> Score 1 (Absence of standing deadwood) <input type="checkbox"/> Score 2 (Presence of standing deadwood)	Results from associated parameters <input type="checkbox"/> Score 1 (Total absence of deadwood) - 2 point <input type="checkbox"/> Score 2 (Presence of standing deadwood as Course woody debris) - 3 point <input type="checkbox"/> Score 3 (Presence of various type of deadwood) - 4 point
		Course woody debris	<input type="checkbox"/> Score 1 (Absence of Course woody debris) <input type="checkbox"/> Score 2 (Presence of Course woody debris)
	Species diversity indicators		
Biodiversity conservation is a key objective for sustainable forest management, but the multi-dimensional and multi-scale character of biodiversity renders full assessment difficult at large scale. Therefore, indicators are often used to monitor biodiversity. The quantity and quality of deadwood are commonly used in nature conservators as indicators of forest biodiversity. Additionally, the presence of species different from the target species as well as the presence of specific type of microhabitat are also used to monitor biodiversity in forest stand.	Species richness (presence of individual of non-target species)	<input type="checkbox"/> Score 1 (Absence of other species) <input type="checkbox"/> Score 2 (Presence of other species)	Results from associated parameters <input type="checkbox"/> Score 1 (Monospecific site) - 2 point <input type="checkbox"/> Score 2 (Sporadic species with some or low regeneration) - 3 point <input type="checkbox"/> Score 3 (Sporadic species with high regeneration) - 4 to 6 point
	% of non-target individuals in the smallest diameter class (10 cm)	<input checked="" type="checkbox"/> Score 1 (%Rin < 15) <input type="checkbox"/> Score 2 (15 < %Rin < 30) <input type="checkbox"/> Score 3 (30 < %Rin < 50) <input type="checkbox"/> Score 4 (%Rin > 50)	
	Microhabitat indicators		
	% of individuals with cavities	<input checked="" type="checkbox"/> Score 1 (%Ind < 10%) <input type="checkbox"/> Score 2 (10% < %Ind < 50%) <input type="checkbox"/> Score 3 (%Ind > 50%)	Results from associated parameters <input checked="" type="checkbox"/> Score 1 (Absence of low number of microhabitat) - 3 point <input type="checkbox"/> Score 2 (Sporadic to consistent presence of microhabitat) - 4 to 6 point <input type="checkbox"/> Score 3 (High presence of microhabitat) - 7 to 9 point
% of individuals with injuries and wounds	<input checked="" type="checkbox"/> Score 1 (%Ind < 10%) <input type="checkbox"/> Score 2 (10% < %Ind < 50%) <input type="checkbox"/> Score 3 (%Ind > 50%)		
% of individuals with deformation	<input checked="" type="checkbox"/> Score 1 (%Ind < 10%) <input type="checkbox"/> Score 2 (10% < %Ind < 50%) <input type="checkbox"/> Score 3 (%Ind > 50%)		
Final Score: 1			

Figure 2.6.2. Forest population assessment form structure.

Forest population assessment sheet - Simulation Site 07 (Tre Termini)				
Forest management	Real Data		Implication for Management	
Forests should be managed in a way to preserve their multifunctional role (ecological, social and productive forest functions). This can be achieved only through maintenance of healthy forests and their biodiversity, protection of its natural fertility and water sources as well as other beneficial functions of forests in the water and carbon cycle, sustainable supply of wood and other products from forest, profit and employment.	Single tree selection thinning			
Forest structure indicator	Real Data	Indicator-specific score		Implication for Horizontal and Vertical Forest structure
Forest structure is both a product and driver of ecosystem processes and biological diversity. Changes in forest structure as a result of management for timber production have undesirable consequences for other components of forest ecosystems	Structural indicator			
	DBH Standar deviation	13.1	<input type="checkbox"/> Score 1 (DBH SD < 10) <input checked="" type="checkbox"/> Score 2 (10 ≤ DBH SD ≤ 20) <input type="checkbox"/> Score 3 (DBH SD > 20)	Results from annotated parameters <input type="checkbox"/> Score 1 (Simple forest structure without rinoovation) - From 4 to 6 <input type="checkbox"/> Score 2 (Simplified forest structure with rinoovation or Complex forest structure without rinoovation) - From 7 to 8 <input type="checkbox"/> Score 3 (Complex forest structure with rinoovation) - From 9 to 11 <input checked="" type="checkbox"/> Score 4 (Complex forest structure with rinoovation present in the total area) - From 12 to 13
	Refers to % of trees in regeneration layer (considering DBH less than 10 cm)	%19	<input type="checkbox"/> Score 1 (% Rim < 15) <input type="checkbox"/> Score 2 (15 ≤ % Rim < 30) <input type="checkbox"/> Score 3 (30 ≤ % Rim < 50) <input checked="" type="checkbox"/> Score 4 (% Rim ≥ 50)	
	Curve of DBH class distribution	J-shaped	<input type="checkbox"/> Score 1 (Bell-shaped) <input type="checkbox"/> Score 2 (Multi-modal) <input checked="" type="checkbox"/> Score 3 (J-shaped)	
N° of strata (stratification of population)	Tri-stratified	<input type="checkbox"/> Score 1 (Mono-stratified) <input type="checkbox"/> Score 2 (Bi-stratified) <input checked="" type="checkbox"/> Score 3 (Tri or Multi-stratified)		
Productivity indicators	Real data		Implication for population biodiversity	
Forest site productivity is the production that can be realized at a certain site with a given genotype and a specified management regime. Site productivity depends both on natural factors inherent to the site and on management-related factors.	Growing stock (m³/ha)	300-400 m³/ha	In managed stands, the minimum growing stock should be around 300-350 m³/ha. This might be applicable for shade tolerant species	
Biodiversity indicators	Real data	Indicator-specific score		Implication for stand biodiversity
Biodiversity conservation is a key objective for sustainable forest management, but the multi-dimensional and multi-scale character of biodiversity renders full assessment difficult at large scale. Therefore, indicators are often used to monitor biodiversity. The quantity and quality of deadwood are commonly used in nature conservation as indicators of forest biodiversity. Additionally, the presence of species different from the target species as well as the presence of specific type of microhabitat are also used to monitor biodiversity in forest stand.	Deadwood indicators			
	Standing deadwood (including snags) presence	Presence	<input type="checkbox"/> Score 1 (Absence of standing deadwood) <input checked="" type="checkbox"/> Score 2 (Presence of standing deadwood)	Results from annotated parameters <input type="checkbox"/> Score 1 (Total absence of deadwood) - 2 point <input checked="" type="checkbox"/> Score 2 (Presence of standing deadwood Coarse woody debris) - 3 point <input type="checkbox"/> Score 3 (Presence of various type of deadwood) - 4 point
	Coarse woody debris	Absence	<input checked="" type="checkbox"/> Score 1 (Absence of Coarse woody debris) <input type="checkbox"/> Score 2 (Presence of Coarse woody debris)	
Species diversity indicators	Real data	Indicator-specific score		
Species richness (presence of individual of non-target species)	Presence	<input type="checkbox"/> Score 1 (Absence of other species) <input checked="" type="checkbox"/> Score 2 (Presence of other species)	Results from annotated parameters <input type="checkbox"/> Score 1 (Monospecific site) - 2 point <input checked="" type="checkbox"/> Score 2 (Sporadic species with none or low regeneration) - 3 point <input type="checkbox"/> Score 3 (Sporadic species with high regeneration) - 4 to 6 point	
% of non-target individuals in the smallest diametric class (10 cm)	2,6	<input checked="" type="checkbox"/> Score 1 (% Rim < 15) <input type="checkbox"/> Score 2 (15 ≤ % Rim < 30) <input type="checkbox"/> Score 3 (30 ≤ % Rim < 50) <input type="checkbox"/> Score 4 (% Rim ≥ 50)		
Microhabitat indicators	Real data	Indicator-specific score		
% of individuals with cavities	17,9	<input type="checkbox"/> Score 1 (% Ind. ≤ 15%) <input checked="" type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%) <input type="checkbox"/> Score 3 (% Ind. > 50%)	Results from annotated parameters <input type="checkbox"/> Score 1 (Absence of low number of microhabitat) - 3 point <input checked="" type="checkbox"/> Score 2 (Sporadic to consistent presence of microhabitat) - 4 to 6 point <input type="checkbox"/> Score 3 (High presence of microhabitat) - 7 to 9 point	
% of individuals with injuries and wound	9,1	<input checked="" type="checkbox"/> Score 1 (% Ind. ≤ 15%) <input type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%) <input type="checkbox"/> Score 3 (% Ind. > 50%)		
% of individuals with deformation	12,5	<input checked="" type="checkbox"/> Score 1 (% Ind. ≤ 15%) <input type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%) <input type="checkbox"/> Score 3 (% Ind. > 50%)		
				Final Score: 10

Figure 2.6.3. Forest population assessment form structure with Site 07 - Tre Termini.

2.7 Recommendations for Sustainable Forest Management

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Overview of silvicultural systems

The silvicultural practices currently applied in *Abies alba* Mill. stands in the mediterranean area vary from a uniform shelterwood system to an irregular shelterwood approach and a system of individual tree selection. In areas where *A. alba* stands originated from planting, such as in the Apennine mountains in Italy, clearcutting with artificial regeneration is used. However, forest management practices with moderate intensity prevail. Regeneration is often natural, stands are selectively thinned, cut sizes in final cuts are small (i.e. < 1 ha). Silvicultural systems that are most suitable for silver fir stands are selection system (individual tree selection) and irregular shelterwood system (group selection system) on small areas. In such conditions, fir can compete with beech, spruce, maples and other tree species in mixed forest stands (ZGS, 2021).

In a individual tree selection system with continuous felling in intervals from 5 to 15 years an optimal wood stock and stand conditions are maintained that are favourable for the regeneration of fir trees. In such forests, the felling amount is approximately equal to the wood increment (Wolf *et al.*, 2010).

In an irregular shelterwood system (group selection system) a longer regeneration period (>30 years) is needed for the successful regeneration and overgrowth of fir trees. This means that in places where we want to promote fir trees, we regenerate and regulate the light conditions gradually and in the long term, by gradually removing the trees in overstory. In areas where regeneration goals are different, regeneration is possible in a shorter period and also in a larger area. Such a method requires careful and differentiated forest management planning. Continuous felling and permanent, but spatially limited, regeneration is also important in an irregular shelterwood system (group selection system).

From this point of view, management focused on intensive increase of growing stock, as well as an intensive regeneration on over large, is not suitable for the fir stands. It is possible to contribute to the regeneration of fir trees by preserving vital (younger) overstory of fir trees in areas regenerated with beech (Wolf *et al.*, 2010).

Due to the considerable differences in the growth of fir trees, as well as in the ecology of regeneration, guidelines regarding silviculture guidelines, cutting cycles and target dimensions have to be adapted to forest type, site and stand conditions. Compared to beech stands, however, the production age and target dimensions in silver fir stands are generally higher than in beech stands due to the growth dynamics (ZGS, 2021).

In LIFE SySTEMiC project four main SFM systems have been studied, from unmanaged to individual tree selection system, irregular and uniform shelterwood system as well as silver fir forests corresponding to four European Forest Types.



Figure 2.7.1. Selection and irregular shelterwood silvicultural systems are the most suitable for management of silver fir stands.

Silvicultural characteristics of Silver fir

Occurrence of silver fir in forest stands is limited with late frosts, drought, summer heat and winter cold. The main advantage of the silver fir in comparison with other forest tree species is in its requirement for light. It is a shade tolerant species, and it is far more effective in unfavourable light conditions than its competitors. Young growth of silver fir can survive long periods in a deep shade of a selection forest. Fir is on the other hand very sensitive regarding warmth and moisture and belong to the species with narrow ecological valency. Further, silver fir is very susceptible to late frost, lateral shoots are usually damaged since they appear earlier in spring (Prpić (ed.), 2001).

Water requirements of silver fir are quite high, and it is very sensitive to drought, especially at a young age. On the other hand, in some cases silver fir surprising tolerance to drought has been observed, especially in central and southern Europe (Carrer *et al.*, 2010). The soils of silver fir sites are very diverse in all their characteristics. It grows on limestone as well as on silicate bedrock (Prpić (ed.), 2001).

Threats

Due to negative consequences of climate change silver fir will most likely be among the more threatened tree species, especially due to the special requirements regarding stand climate and difficulties in natural and artificial regeneration caused by the game browsing. The share of silver fir in forests and thus the number of populations and/or the density of fir trees has been decreasing for several decades. Small populations or populations with sparsely planted trees are subject to genetic drift and self-fertilization and a consequent decrease in the fitness of the population and their genetic diversity (Wolf *et al.*, 2010). Decline in the proportion of fir is faster on fir-beech sites than on fir-spruce

and pure fir sites, where regeneration is often more successful, browsing damage is smaller, and the age classes structure promises more successful preservation of fir in future forests. Predictions about the reduction of the fir share in forest communities in future scenarios, where an increase in temperatures and a decrease in the amount of precipitation are predicted, are also worrying, especially in the combination with difficulties in regeneration (Wolf *et al.*, 2010).

In the 1970s and 1980s widespread decline and even dieback of silver fir was observed in Central Europe, a phenomenon referred to as “fir dieback”. There was a range of opinions regarding the reasons for fir mortality, but the prevailing view was that growth depression of fir between 1970 and 1990 was caused by SO₂ in a complex interaction with climatic and biotic factors (Abies, 2016).

Climate change is believed to have had an adverse impact on the growth performance of autochthonous fir populations in Europe in the last decades. The main threat posed by climate change is that in the possibility of negative impacts from abiotic and biotic (pests, diseases) factors is increasing. A decrease in the proportion of fir due to white rot (*Sclerotinia sclerotiorum*) is also expected, as a result of the sudden opening of a large area (large-scale felling due to natural disasters) and thus a rapid change in the microclimate. There is also a danger of weeding of regeneration gaps with non-native invasive plant species and thereby of limiting the possibility of natural rejuvenation of native species. Due to the invasion of non-native invasive plant species, the properties of the soil will change and deteriorate, the impoverishment of habitats will increase, biodiversity will decrease, and the predation of autochthonous young growth by herbivorous game will increase (ZGS, 2021).

Assessment of adaptation potential of Silver fir forests to climate change

The adaptation potential of the silver fir forests depends on forest site, stand structure and tree species composition. Due to the appropriate structure of the forests (group selection and selection structure), the appropriately structured forest edge, the presence of selective thinning of stands, implemented medium-term thinning of stands, a high degree of preservation of tree composition and fairly undisturbed natural rejuvenation it can be very high. Adaptation potential is strongly reduced by the low level of implementation of tending measures in the younger developmental stages, by uniform stand structure, by altered tree species composition and by the excessive browsing of game (ZGS, 2021).

Regeneration of Silver fir stands

Due to the long regeneration periods in fir stands, it is necessary to start with the regeneration process earlier than in the case of tree species with short regeneration periods. Large differences in the growth of trees that are the same dimensions, point to the need to decide on felling at the level of individual trees (ZGS, 2021).

The restoration of stands should take place on small regeneration areas, as this enables the rejuvenation of key tree species in particular. Even the needs for the tending measures of young growth are reduced with small-area approach. The size of the gaps regulates the composition of the young growth: lower intensities provide a greater proportion of fir, more light is needed by spruce, and even more deciduous trees apart from beech, which is a shade-tolerant species. In the drier areas, it makes sense to open larger areas to ensure the restoration of more drought tolerant species such as pine and larch, which can substitute silver fir and beech. At the same time when regeneration felling is executed, it is necessary to remove non-vital trees in understorey and shrubs.

Problems with regeneration can occur with large-scale restorations after natural disasters. In these cases, it is necessary to intensify the preparation of the stand for natural regeneration, and in some cases also to intervene with regeneration through planting. Planting is also used where it is not possible to provide a suitable proportion of deciduous trees naturally.

On non-carbonate forest sites, where silver fir is the dominant species, there are no problems with regeneration. Here, we only preserve its natural share in tree composition. On sites with carbonate bedrock, for example on high karst in Slovenia, in fir-beech forests, the beech is once again increasing its share in the anthropogenically modified tree structure in cyclical succession. Here, among all tree species, silver fir is the most endangered by browsing of herbivorous game. In the light conditions in which fir competes with other species, it grows relatively slow and is therefore exposed to browsing for a longer period. In such conditions, without special attention given to fir, we cannot expect a further increase in its share, which has been steadily decreasing in recent decades. On such sites, we direct the development of the forest through natural forest regeneration, where the fir trees are successfully regenerated in fenced areas and/or by planting fir trees. The individual protections of seedlings from game browsing are also important (ZGS, 2021)



Figure 2.7.2. In some areas protection against ungulate browsing is essential for silver fir regeneration.

Tending and protection of Silver fir stands

The most appropriate maintenance and protection measures are summarized based on of SFM guidelines in Slovenia (ZGS, 2021) and the LIFE SySTEMiC results. The most common tending measure in young growth is the gradual removal of shrubs and overgrowths while simultaneously regulating the mixture of target tree species in forest stands. In individual tree selection forests tending measures

concentrated on smaller groups of young growth. With tending measures in young growth, we create a varied vertical and horizontal structure of the stands and thereby the stability of forests against harmful abiotic influences (wind, frost, sun, wet snow).

It is important to shorten the production period in fir stands (cutting down a fir tree when its growth begins slow down, i.e. between 80 and 100 years).

Productive silver fir sites require more intensive thinning, especially of younger development stages (between 20 and 25%). Thinning must be early and aimed at regulating the tree composition and strengthening the stability of the stands. During thinning, particular attention should be paid to ensuring the stability of stands and the preservation of deciduous trees in the drier parts of the forest stand. Thinning of medium aged stands should range between 15 and 20%.

In selection forests felling should be aimed at maintaining the selection forest structure.

In mature stands the thinnings intensity should be lower (between 10 and 15% of growing stock) and shouldn't produce larger gaps in forest stands.

A sufficient proportion of silver fir in future forests will be ensured primarily by controlling the light conditions on forest floor. The species mixture of the main tree species should be in groups, and the admixture of noble deciduous trees should be individual or group. In addition to the main tree species, the understory layer is also important.

An important measure is the conversion of one-dimensional stands into more structured ones by means of selective thinning. Conversion is carried out in stands built by tree species that are unsuitable for the given forest site (plantations of spruce for example), namely when the vitality of the stands or the state of health of the stands deteriorates so much that it threatens the normal forest management.

These weakened and non-vital stands must first be thinned out and thereby ensure a greater inflow of light, which leads to the natural formation of an understory layer of meliorative tree and shrub species, which will improve the properties of the soil. This will ensure that the stands will develop in the direction of potential vegetation in a progressive successional process. During the conversion, special attention is paid to minority tree species, which have a meliorating ability, which indirectly improves and increases forest production. Particularly important are the tree species that combine the melioration function and at the same time have an economic value (beech, sycamore maple, mountain elm, large ash, linden, hornbeam, wild cherry, wild pear, larch, chestnut, black alder, aspen, etc.).

A balanced structure of individual tree selection stands is much more resistant to negative abiotic factors than a uniform structure. Therefore, it makes sense to convert uniform stands into selection ones by means of selection thinnings. Transformation by thinning should be started as early as possible, release the crowns to the selected ones and create a network of trees that supports the stability of the stands.

Forest protection measures in silver fir stands mostly consists of game browsing protection with repellents or individually. Protection with a fence is used mainly in areas with a higher concentration of game. Sanitary felling should be regular and quick - all heavily attacked trees by pests and diseases (e.g. white mistletoe, frostbite and fir canker) should be removed. In individual tree selection stands it is important to take care of a balanced distribution of trees by diameter classes, thereby preventing the silver fir from the loss drying out in stands with an excessive proportion of thick trees (thickness class over 50 cm).

Adaptation of Silver fir stands to climate change

Among the most important measures, with which we can contribute to the preservation of fir trees in climatically unstable environment are:

- selective thinning, which can enable the social rise of fir trees in even-aged stands,
- tending measures in younger stands in which we can increase the number of fir trees by regulation of mixture and later by positive selection,
- planting of fir trees under the canopy (e.g. in spruce cultures) and
- maintaining gaps within forests, maintaining a structured forest edge and sufficient proportion of seed trees.

Genetic variability of silver fir is one of the most important factors in its response to climate change (Oggioni, 2024) since the adaptability and growth characteristics of trees can vary depending on their origin. SFM of silver fir stands should therefore support their natural migration and adaptation process by assisted migration, by planting of mixed selected provenances in the most favourable places for the future.



Figure 2.7.3. Silver fir rejuvenation in selection stands.





3. SUSTAINABLE FOREST MANAGEMENT OF *FAGUS SYLVATICA* L.

3.1 Introduction

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The European beech (*Fagus sylvatica* L.) is a wind pollinated monoecious species with a life span of up to 300 years, reaching the height of 40 to 60 m. It is a stand-forming species growing in pure or mixed stands throughout Europe. In the south-east it may interbreed with *Fagus orientalis* Lipsky (Figure 3.1.1). It grows on a wide range of soils, except compacted and waterlogged ones, and prefers humid climates. Since it is susceptible to high temperatures, summer droughts and late frosts, its distribution is limited in the Mediterranean and in continental parts of the western and northern Europe (Westergren *et al.*, 2020).

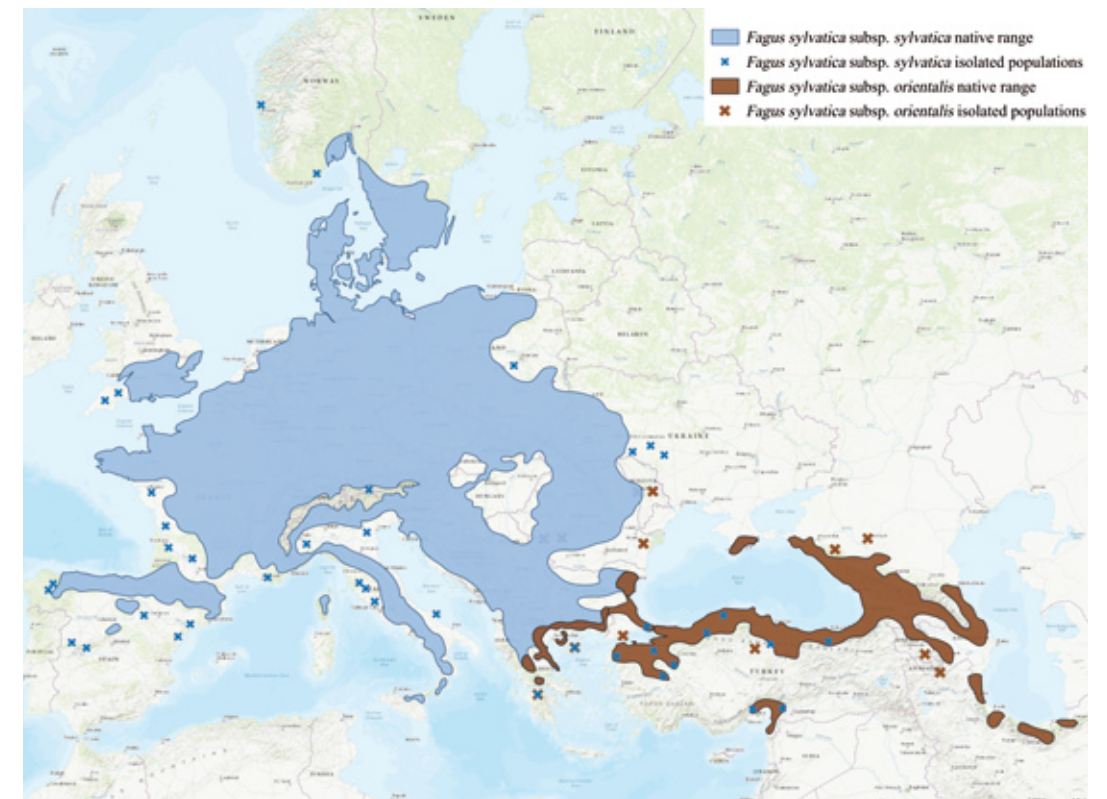


Figure 3.1.1. European beech distribution range (www.euforgen.org) by Caudullo G., CC BY 4.0 <<https://creativecommons.org/licenses/by/4.0/>>, via Wikimedia Commons.

The European beech reaches its reproductive phase at 50 to 60 years of age. Separate male (catkins) and female flowers are found on the same branches and the same buds. Flowering follows immediately leaf budburst in April and May. Beech three-angled acorns, held as 2 to 3 in cupules, ripen in September - October. The ripe brown acorns drop from trees till the end of November. A full mast year normally occurs periodically every 5 to 10 years, while some trees may flower every few years.

Fresh acorns have 20-30% water content, their dormancy varies, and the cold stratification may last from 4 to over 20 weeks. When dried to 8-9% it can be stored at -10 to -15 °C for 5 to 6 years or more at artificially elevated CO₂ concentration. In 1 kg of fresh beech nuts (at 20-30% humidity) there can be 3000-6000 nuts, while 1000 nuts weigh approximately 150-300 g. First nuts that fall in September are usually empty due to insects (Kraigher, 2024; Regent, 1980; USDA 2008).

Westergren *et al.* (2020, with references therein) note that spring frosts often damage young trees or flowers that appear at the same time as leaves. *Mikiola fagi* Hartig can kill young beech trees and reduce increment in heavily infested trees. Beech is also among the susceptible hosts of *Phytophthora ramorum* Werres, De Cock & Man, a quarantine oomycete. *Anoplophora chinensis* Forster, the citrus long-horned beetle and *Anoplophora glabripennis* Motschulsky, the Asian long-horned beetle, both originating from Asia, pose a new threat for beech.

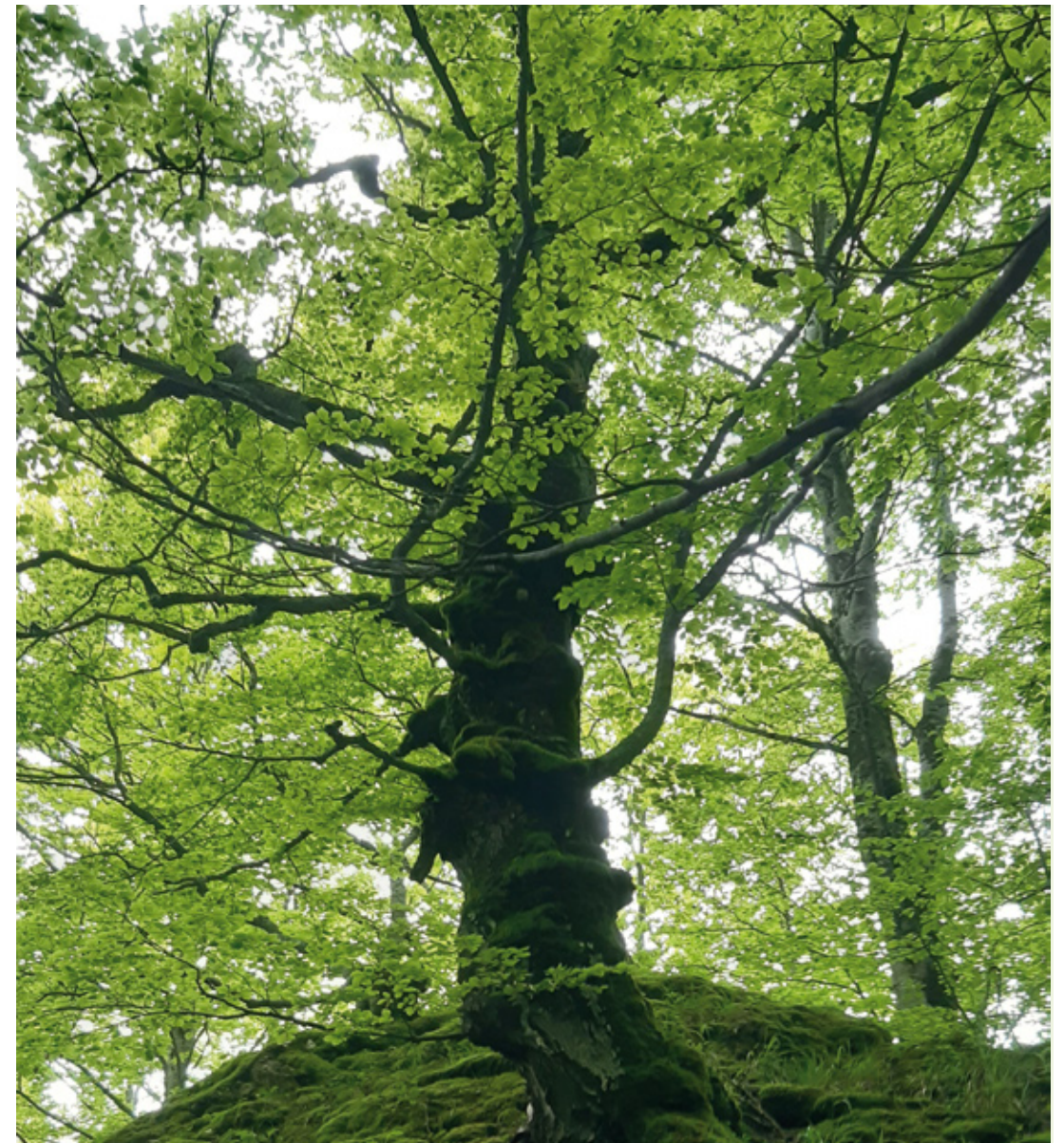
The European beech is characterised by a low genetic structure across the European continent. The populations of the Iberian Peninsula and south-west France, the Italian Peninsula and the south-eastern Balkans (there may be other gene pools in the region) each belong to different gene pools, while the central European gene pool mixes with the Balkan gene pool and expands towards the northern range of the species (Demesure *et al.*, 1996; Magri *et al.*, 2006; GenTree, 2021; Höhn *et al.*, 2021), with isolation by distance playing an important role in differentiation (Höhn *et al.*, 2021). The genetic diversity of beech appears to be highest in glacial refugia (GenTree, 2021). Overall, populations in the centre of the range are more similar in terms of diversity and structure (GenTree, 2021). Table 3.1.1 reports the list of the sites for *Fagus sylvatica* L. of the LIFE SySTEMiC project.

Table 3.1.1 List of the sites for *Fagus sylvatica* L. of the LIFE SySTEMiC project.

Id	Site name	Country	Species	EFT*	Structure	Silvicultural system
01	Pian degli Ontani	Italy	<i>F. sylvatica</i>	7.3	Even-aged	Uniform shelterwood
02	Baldo's forest	Italy	<i>F. sylvatica</i>	7.3	Uneven-aged	Individual tree selection
03	Pian dei Ciliegi	Italy	<i>F. sylvatica</i>	7.3	Even-aged	Uniform shelterwood
04	Caselle 1	Italy	<i>F. sylvatica</i>	7.3	Even-aged	Uniform shelterwood
05	Caselle 2	Italy	<i>F. sylvatica</i>	7.3	Even-aged	Uniform shelterwood
11	Fonte Novello	Italy	<i>F. sylvatica</i>	7.3	Uneven-aged/Old-growth	Unmanaged
12	Venacquaro	Italy	<i>F. sylvatica</i>	7.3	Even-aged	Uniform shelterwood
14	Ogulin	Croatia	<i>F. sylvatica</i>	7.2	Even-aged	Uniform shelterwood
23	Osankarica	Slovenia	<i>F. sylvatica</i>	7.2	Even-aged	Irregular shelterwood

24	Pri Studencu	Slovenia	<i>F. sylvatica</i>	6.6	Even-aged	Irregular shelterwood
25	Rajhenavski Rog	Slovenia	<i>F. sylvatica</i>	7.4	Uneven-aged/Old-growth	Unmanaged
29	Gorski kotar, Vrbovsko	Croatia	<i>F. sylvatica</i>	7.2	Uneven-aged	Individual tree selection

* EFT = European Forest Type: 6.6 Illyrian submountainous beech forest; 7.2 Central European mountainous beech forest; 7.3 Apennine-Corsican mountainous beech forest; 7.4 Illyrian mountainous beech forest.



3.2 Forest structure deadwood and tree-related microhabitats

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Forest structure, deadwood and tree-related microhabitats were inventoried in 11 sites (Figure 3.2.1).

A permanent observation plot with a representative structure of forest management was selected at each site. The plot had a rectangular shape and each side was 50 m long (area of 2500 m²). Only when the number of trees of the target species (*Fagus sylvatica* L. with diameter at breast height > 2.5 cm) was < 30 trees one more plot was established in the site, until the minimum number of 30 trees was reached.

Detailed protocols for field survey and data elaboration are available on LIFE SySTEMiC web page: <https://www.lifesystemic.eu/>.

The spatial distribution of the trees in the sites is reported in Figure 3.2.2. Figure 3.2.3 shows the stem number-diameter distribution in each site.

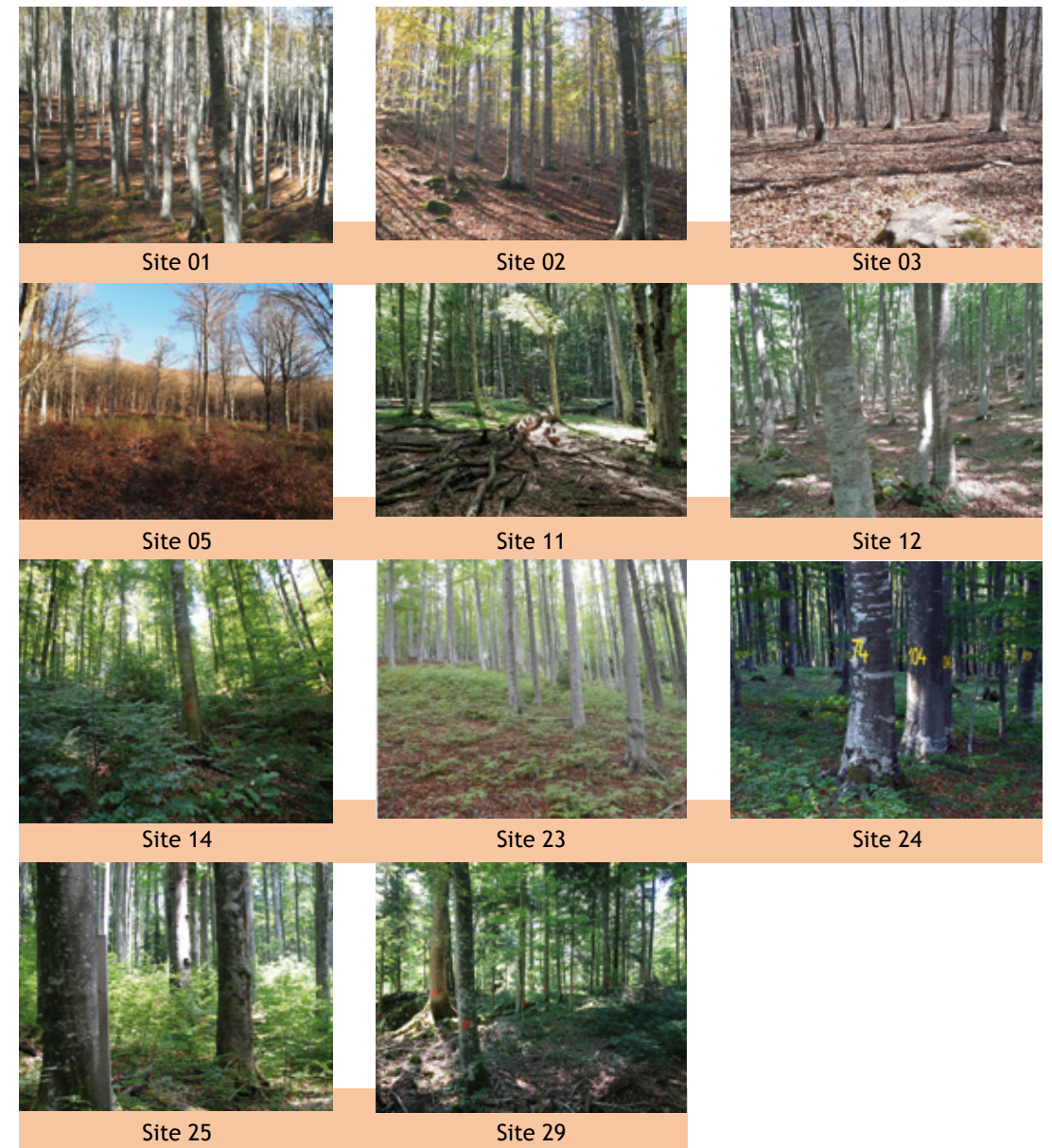


Figure 3.2.1 *Fagus sylvatica* L. was the prevailing tree species (basal area > 75%) in the Sites 01 - Pian degli Ontani, 02 - Baldo's forest, 03 - Pian dei Ciliegi, 05 - Caselle 2, 11 - Fonte Novello, 12 - Venacquaro, 14 - Ogulin, 23 - Osankarica, 24 - Pri Studencu; in the remaining Sites *Fagus sylvatica* L. was most often mixed with *Abies alba* Mill. (Sites 25 - Rajhenavski Rog and 29 - Gorski kotar, Vrbovsko), *Acer pseudoplatanus* L., *Tilia platyphyllos* Scop. and other secondary tree species (Site 29).

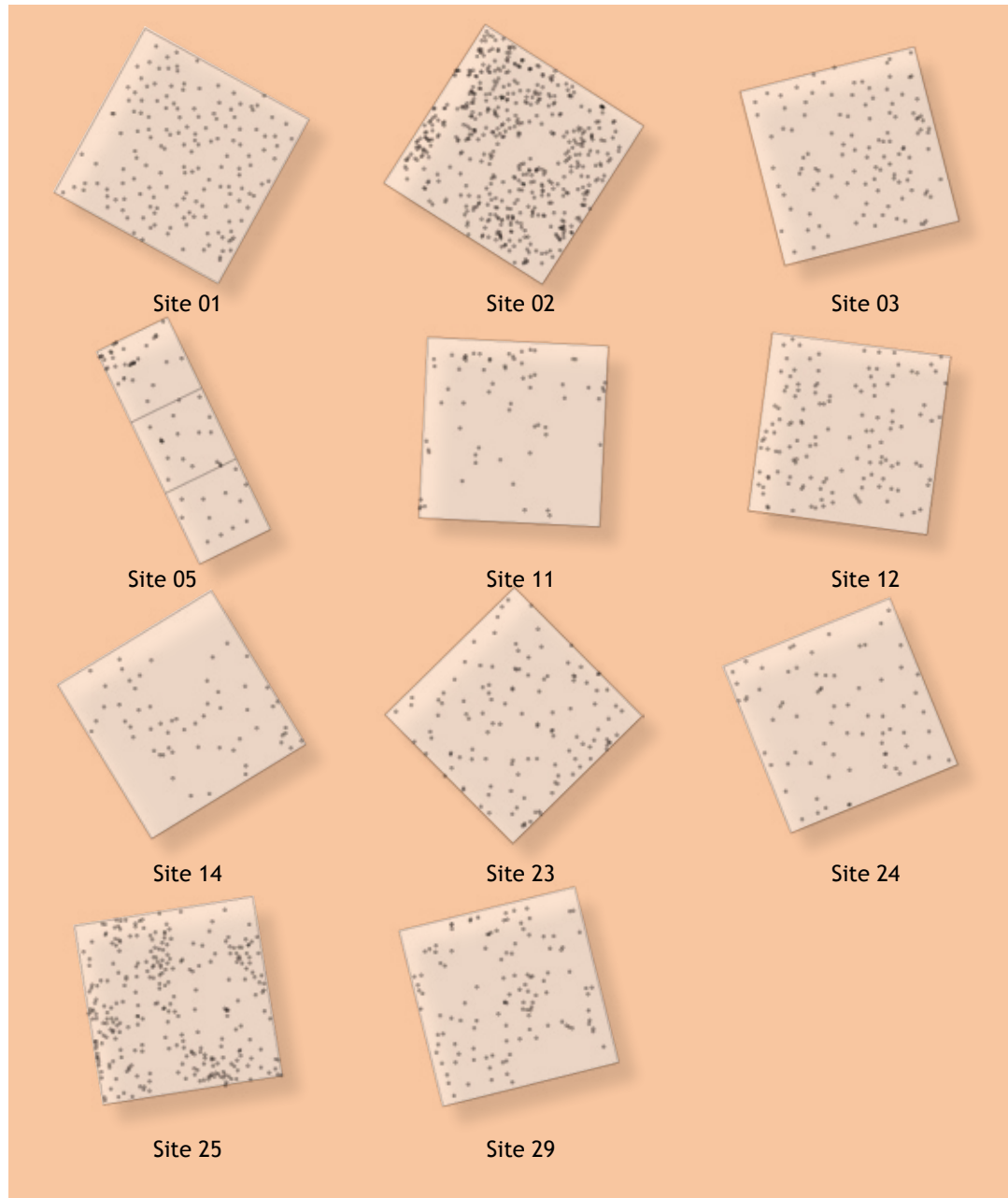


Figure 3.2.2. Spatial distribution of the trees in the sites.

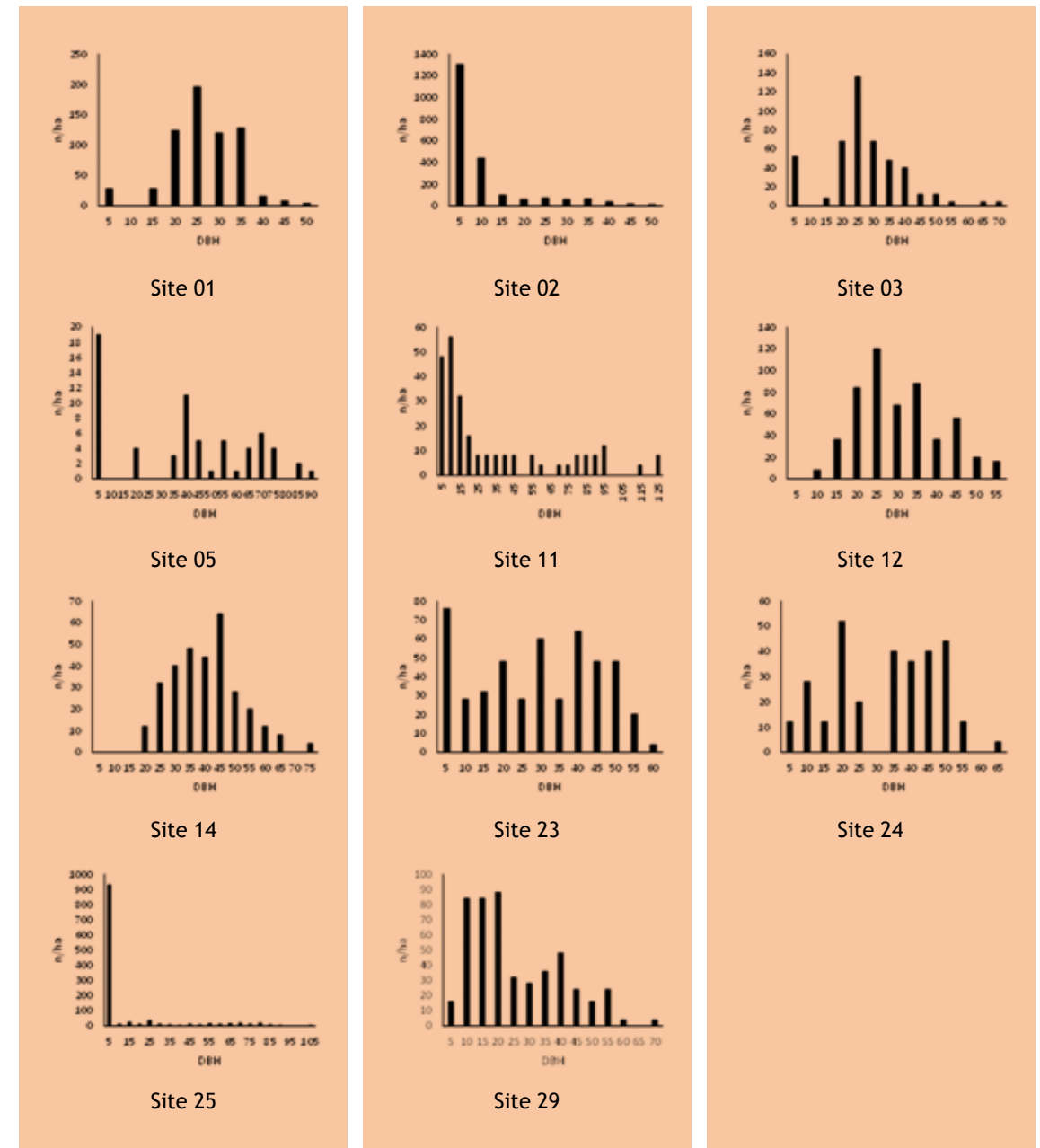


Figure 3.2.3. Stem number-diameter distribution in the sites.

Forest structure, deadwood and tree-related microhabitats were inventoried in 11 sites (Figure 3.2.1).

Forest structure variables are reported in Table 2.2.1. In the examined *Fagus sylvatica* L. stands, the basal area ranged between 15 m²/ha and 55 m²/ha and the volume varied between 204 m³/ha and 987 m³/ha. In managed even-aged stands (Sites 1 - Pian degli Ontani, 3 - Pian dei Ciliegi, 5 - Caselle 2, 12 - Venacquaro, 14 - Ogulin, 23 - Osankarica, 24 - Pri Studencu), the basal area was 35 m²/ha and the volume was 490 m³/ha (average values); in managed uneven-aged stands (Sites 2 - Baldo's forest, 29 - Gorski kotar, Vrbovsko) the basal area was 33 m²/ha and the volume was 354 m³/ha (average values). The old-growth stands (Sites 11 - Fonte Novello, 25 - Rajhenavski Rog) had uneven-aged structures, the basal area was 52 m²/ha and the volume was 904 m³/ha (average values).

Forest structure diversity, represented by the trees dimensional diversity (standard deviation and coefficient of variation of DBH and heights), was high in the old-growth stands (CVDBH = 135.7%, CVH = 82.6%) followed by uneven-aged (CVDBH = 76.0%, CVH = 54.5%) and even-aged (CVDBH = 44.6%, CVH = 31.8%) stands.

Table 3.2.1. Forest structure variables in the sites. N=number of stems; BA=basal area; V=volume; QMD=quadratic mean diameter (diameter of the mean basal area tree); MH= mean height (height of the mean basal area tree); DH= dominant height (mean height of the 100 trees per hectare with the largest diameters in case of even-aged stand, and mean height of the three tallest trees per hectare in case of uneven-aged stand); SDDBH= standard deviation of DBH; SDH= standard deviation of total tree heights; CVDBH= coefficient of variation of DBH; CVH= coefficient of variation of total tree heights.

Id	N	BA	V	QMD	MH	DH	SDDBH	SDH	CVDBH	CVH
	n/ha	m ² /ha	m ³ /ha	cm	m	m	cm	m	%	%
01	652	38.5	528.2	27.4	27.4	28.4	8.1	5.3	30.8	20.1
02	2164	32.6	363.4	13.9	16.2	30.8	9.5	6.9	94.9	65.7
03	456	31.3	340.5	29.6	21.3	22.8	12.3	5.7	45.6	29.6
05	88	15.2	204.4	46.9	25.1	25.6	26.9	10.1	69.7	51.9
11	260	48.0	820.7	48.5	29.6	44.6	34.5	11.3	100.6	55.3
12	532	43.6	639.9	32.3	28.6	31.9	10.3	5.5	33.6	20.4
14	312	42.3	694.3	41.5	30.0	31.6	11.6	3.9	29.0	13.3
23	484	41.2	534.9	32.9	26.3	31.6	16.1	10.7	56.2	50.0
24	300	31.5	489.0	36.5	33.0	37.4	15.7	10.8	47.5	37.4
25	1168	54.9	986.7	24.5	23.4	46.1	21.1	10.3	170.7	109.9
29	488	32.5	344.2	29.1	18.1	28.8	14.5	6.5	57.1	43.3

The amount of deadwood is given for each site in Table 2.2.2. In the stands analysed, the total volume of deadwood ranged between 5 m³/ha and 420 m³/ha. In managed even-aged stands (Sites 01 - Pian degli Ontani, 03 - Pian dei Ciliegi, 05 - Caselle 2, 12 - Venacquaro, 14 - Ogulin, 23 - Osankarica, 24 - Pri Studencu), the total volume of deadwood was 8 m³/ha (average value), which was mostly represented by stumps (58%), other lying deadwood pieces (22%) and snags (20%); in managed uneven-aged stands (Sites 2 - Baldo's forest, 29 - Gorski kotar, Vrbovsko) the total amount of deadwood was 17 m³/ha (average value), that was mostly represented by stumps (51%) and snags (41%). The old-growth stands (Sites 11 - Fonte Novello, 25 - Rajhenavski Rog) had the largest amount of deadwood (329 m³/ha), represented by other lying deadwood pieces (58%), snags (16%), downed dead trees (23%) and stumps (3%).

The frequency of the tree-related microhabitats in each site, represented as the percentage of forms of microhabitats, is reported in Figure 3.2.4. Cavities and deformation / growth form were the most common forms of microhabitats in the Sites 01 - Pian degli Ontani, 02 - Baldo's forest, 03 - Pian dei

Ciliegi, 05 - Caselle 2 and 12 - Venacquaro. Deformation / growth forms were the most common also in Sites 23 - Osankarica and 24 - Pri Studencu. Deadwood and injuries and wounds were well represented in the old-growth stands (Sites 11 - Fonte Novello, 29 - Gorski kotar, Vrbovsko). Epiphytes were common at Site 25 - Rajhenavski Rog.

Table 3.2.2. Volume of deadwood in the sites.

Id	Standing dead trees (including snags)	Downed dead trees	Other lying dead wood pieces	Stumps	Total
	m ³ /ha	m ³ /ha	m ³ /ha	m ³ /ha	m ³ /ha
01	0.0	0.0	2.3	3.0	5.3
02	0.5	0.0	0.0	5.9	6.4
03	0.0	0.0	0.1	5.8	5.9
05	0.0	0.0	0.3	7.1	7.4
11	0.4	0.0	216.6	20.8	237.8
12	3.3	0.0	0.2	1.7	5.2
14	5.2	0.0	4.7	3.4	13.3
23	0.0	0.1	1.5	7.3	8.9
24	2.5	0.0	2.6	2.7	7.8
25	103.0	149.4	163.4	4.2	420.0
29	13.1	2.0	0.8	11.0	26.9

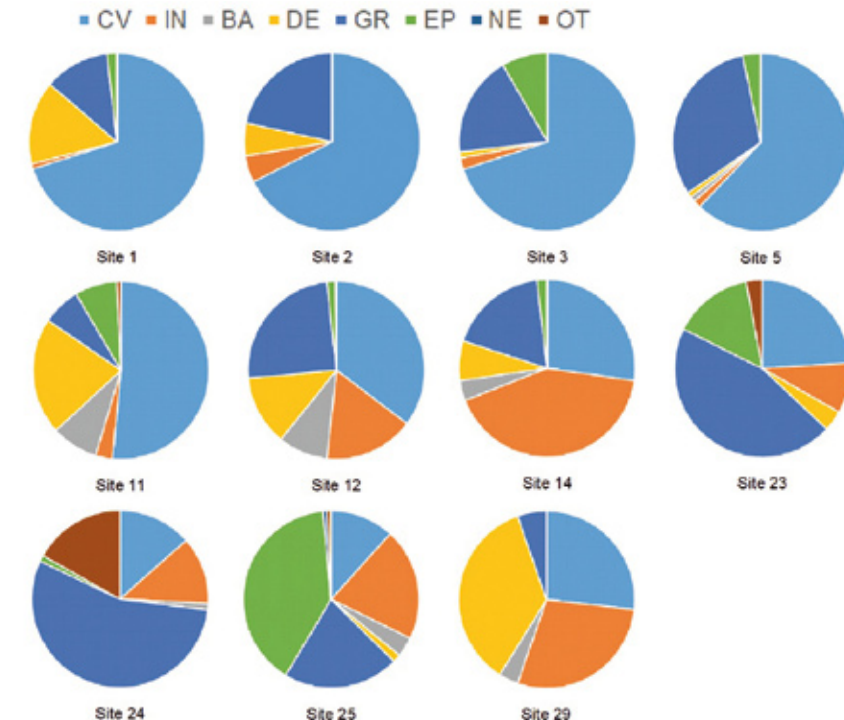


Figure 3.2.4. Tree-related microhabitats in the sites (percentage of forms of microhabitats) (CV = Cavities; IN = Injuries and wounds; BA = Bark; DE = Deadwood; GR = Deformation / growth form; EP = Epiphytes; NE = Nests; OT = Other).



Figure 3.2.5. Natural regeneration of *Fagus sylvatica* L. in the Site 02 - Baldo's forest.



Figure 3.2.6. Deadwood and tree-related microhabitats in the Site 11 - Fonte Novello, old-growth forest.

3.3 Landscape genomics

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Genetic variation is crucial for the fitness and survival of individuals within a population, and it is directly correlated with ability of the species to adapt to different environments (Balkenhol *et al.*, 2016). Within each population, genetic changes, plasticity and evolvability result from the combination of random and selectively oriented processes, which can be influenced by forestry practices (Lefèvre *et al.*, 2014). Understanding the dynamics and mechanisms of local adaptation in natural populations provides basis for predicting responses to environmental changes, including those associated with global climate change. Local adaptation is expected to alter the allele frequencies of genes that influence fitness in particular environments (Lefèvre *et al.*, 2014).

Changes have already been observed in marginal populations of *Fagus sylvatica* L., and several modelling studies predict shifts in the range of this species in the context of global warming. In this context, landscape genomics has emerged as a flexible analytical framework for understanding the interactions between environmental heterogeneity and adaptive genetic variation in natural populations (Balkenhol *et al.*, 2016). We used Landscape Genomics approaches to analyse the neutral and adaptive component of genetic diversity to highlight possible patterns of local adaptation in the populations analysed. Therefore, we correlated adaptive molecular markers (SNPs) with bioclimatic indicators. Nuclear microsatellite markers (nSSR) were analysed as a measure of neutral genetic variation and structure of the studied populations. Single Nucleotide Polymorphism (SNPs) genotyped using a target re-sequencing approach of candidate genomic regions were analysed as a measure of adaptive genetic variation of the studied population (Figure 3.3.1). As a result, for *F. sylvatica* target re-sequencing about 1400 SNPs were observed in 18 genome regions relevant for response to one or more abiotic stresses (results reported in deliverable Action B1: SNP road-map of each study site). In addition, we investigated the distribution of these SNPs at the population level, and we have designed road-maps for each target species to observe their spatial distribution among all the analysed populations (Figure 3.3.1).

A higher number of site-specific and country-specific SNPs can be observed for sites located in Italy (Figure 3.3.1). This frequency of SNPs could be interpreted as a sign of adaptation to a Mediterranean bio-climatic regime that characterizes the Italian peninsula and sets it apart from the more continental climate found in Slovenia and Croatia. As stated in bibliography, analysis for detecting the signature of local adaptation in natural populations requires many analytic steps (Blanquart *et al.*, 2013). The identification of loci with significant correlations with bioclimatic indicators, achieved by evaluating associations between climate variables and allelic variants using different models, prove to be valuable. For each site, twelve bio-climatic indicators were selected from the existing literature (Flint *et al.*, 2013; Gugger *et al.*, 2016, 2021; Pluess *et al.*, 2016). To identify local adaptation patterns of the target species, we conducted GEA analyses on two levels: global and site-specific analysis. The global analysis allowed us to identify possible patterns of adaptation to the bioclimatic conditions that characterize the range of beech. The results of the analysis showed the existence of five different genotypes present in Italy, Croatia and Slovenia. Additionally, an even more interesting finding is the presence of association between 98 allelic variants and the mean values of the 12 bioclimatic indicators considered for these analyses (as reported in Deliverable Action B1: Production of maps of spatial distribution of genetic diversity and of correlation between allele distribution and environmental variation). The presence of these associations could be interpreted as the basal adaptation genotype of beech spread in the Central European range. Of particular interest was finding

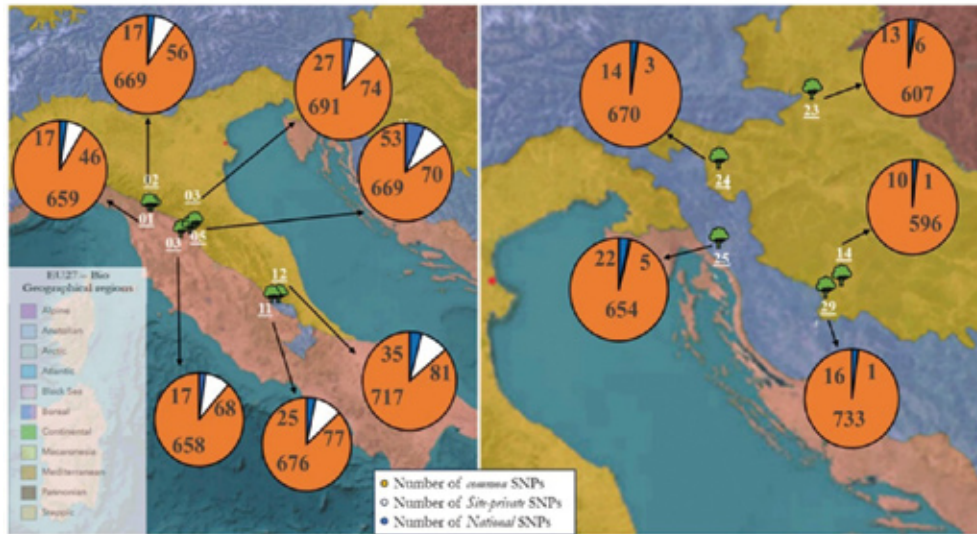


Figure 3.3.1. The road-map of *Fagus sylvatica* L. SNPs distribution in LIFE SySTEMiC sites is depicted in the figure. The figure shows the number of certain specific subsets of SNPs for each site. The data types are sorted by colour. The colours represent the following SNPs subset: The set of SNPs present in that site which are common to at least two sites of interest (orange); the number of unique SNPs specific to that site (white) and the number of SNPs present in that site, which are specific to the country of origin (wblue). Below each graph, the identifying number of the corresponding site is displayed (in bold and underlined).

some site-specific allelic variants associated with a particular country (Italy or Slovenia/Croatia) and individual sites. The presence of allelic variants associated with individual sites could be correlated with the local rather than regional pattern of adaptation. In the environmental association analysis (EAA) it is important to account for neutral genetic structure (Rellstab *et al.*, 2015), as neutral genetic structure can produce patterns like those expected under non-neutral processes (Excoffier and Ray, 2008; Excoffier *et al.*, 2009; Sillanpää, 2011). Furthermore, the genetic structure of populations was analysed using STRUCTURE (Pritchard *et al.*, 2000) and GENELAND software (Guillot 2008).

In general, we found a high number of specific allelic variants in unmanaged sites and in old-growth forests (Figure 3.3.2). The presence of a high number of SNPs associated with adaptation to bioclimatic indicators at these sites could be correlated with the neutral genetic structure observed for these sites (Aravanopoulos, 2018; Paffetti *et al.*, 2012; Stiers *et al.*, 2018). Indeed, some studies have observed that these sites are characterized by a complex neutral genetic structure, characterized by a high number of family clusters. This high variability could provide a higher probability for the emergence of new allelic variants that could enrich the adaptive potential of this species for current and future climate change. A similar situation was observed in stands managed with reduced-impact management types: individual tree selection. Once again, the number of allelic variants specific to each site is high. An extremely interesting finding is the number of allelic variants associated with local adaptation found at the Baldo's Forest site (Figure 3.3.3).

This site has the highest number of SNPs associated with bioclimatic indicators. Similar data were found at other managed sites. Once again, the number of allelic variants observed may be correlated with past silvicultural management. The creation of gaps in forest cover and the complexity of structure characterizing these stands could be linked to a higher probability of gene recombination between genotypes belonging to different family clusters. These patterns are like those found in old-growth forests and unmanaged populations. Thus, the potential for reduced-impact silvicultural

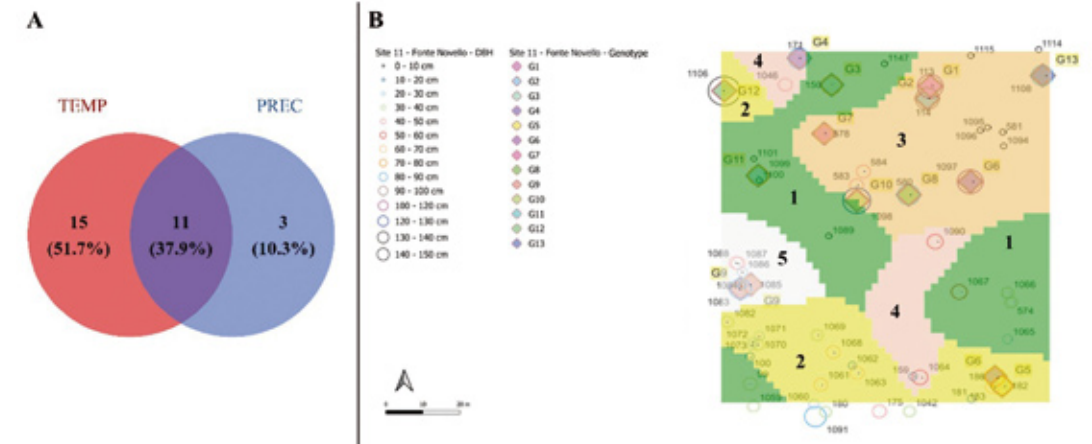


Figure 3.3.2. LFMM analysis results and genotype distribution map of Site 11 - Fonte Novello. (A) Venn diagram showed the overlapping between SNP associated with temperature-related and precipitation-related bioclimatic indicators, as resulted from LFMM analysis. (B) Spatial distribution of genotype and spatial organization in 5 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter are characterized by coloured circles according to the genotype observed. Identical colours mean identical genotypes.

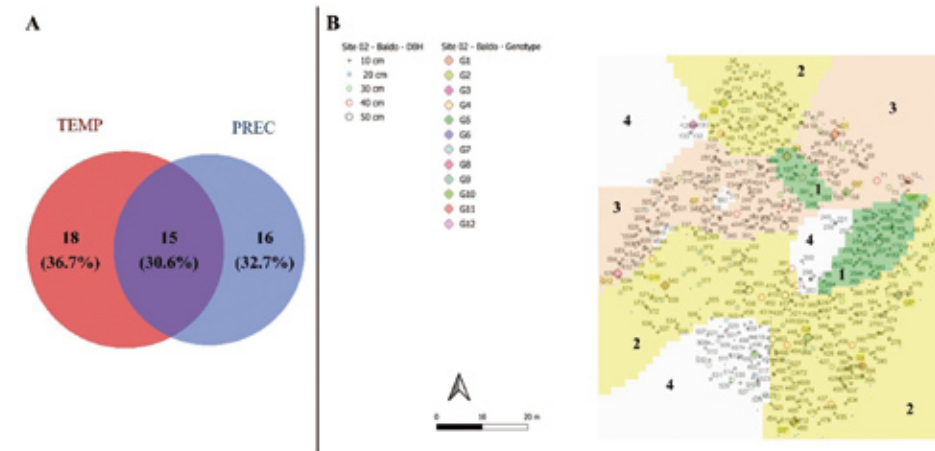


Figure 3.3.3. LFMM analysis results and genotype distribution map of Site 02 - Baldo's Forest. (A) Venn diagram showed the overlapping between SNP associated with temperature-related and precipitation-related bioclimatic indicators, as resulted from LFMM analysis. (B) Spatial distribution of genotype and organization into 4 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter are characterized by coloured circles according to the genotype observed. Identical colors mean identical genotypes.

management to conserve adaptive genetic diversity appears promising. These data are further supported by the analysis of the other beech sites in the study. An intriguing discovery is the presence of the lowest number of specific allelic variants at certain sites. Once again, all these sites share a uniform shelterwood management system (Figure 3.3.4).

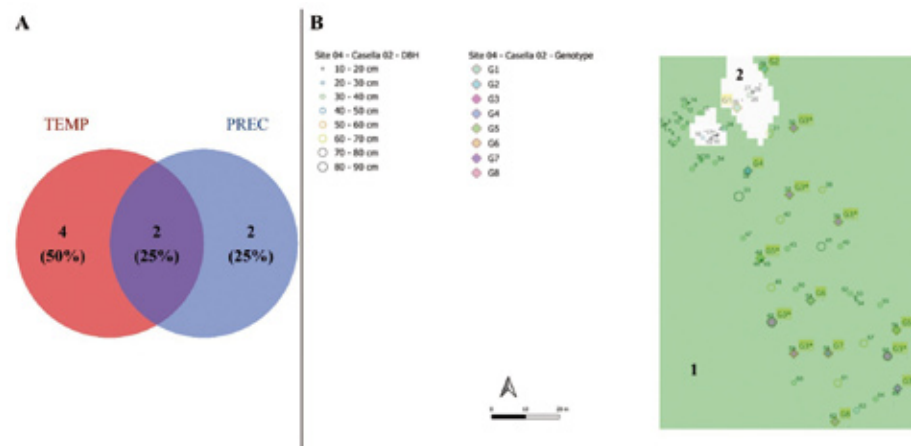


Figure 3.3.4. LFMM analysis results and genotype distribution map of Site 05 - Casella 02. (A) Venn diagram showed the overlapping between SNP associated with temperature-related and precipitation-related bioclimatic indicators, as resulted from LFMM analysis. (B) Spatial distribution of genotype and spatial organization in 2 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter are characterized by coloured circles according to the genotype observed. Identical colors mean identical genotypes.

This type of forest management was developed to promote natural regeneration. However, previous studies (Aravanopoulos, 2018; Brang *et al.*, 2014; Paffetti *et al.*, 2012; Stiers *et al.*, 2018) have shown how the spatial genetic structure that characterizes this type of stand is rather simple. The presence of a simple spatial genetic structure, in fact, might be associated with a lower rate of gene recombination, thus reducing the possibility of creating new allelic variants and favouring inbreeding. This can lead to high levels of homozygosity and inbreeding depression. Less impactful management types, such as individual tree selection, appear to report population with a high number of allelic variants associated with response to bioclimatic indicators. Similar results have been observed in unmanaged stands and old-growth forests. The results reported in this study could play an instrumental role in silvicultural management planning, where knowledge of genetic variability from an adaptive perspective could help in decision-making processes. In addition, this knowledge could also be used in anticipation of assisted migration strategies. It is important to preserve the current Forest Genetics Resources (FGR), but also to enrich the existing stand with potentially favourable genotypes.

3.4 Soil biodiversity

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Ectomycorrhizal root tips support specific and diverse populations of bacteria and microfungi, including bacteria that help to establish mycorrhizal symbioses. Tree roots also harbor dark septate endophytes and wood-decomposing fungi (Baldrian, 2017). Besides mycorrhizal mycelia and associated organisms, bulk soil hosts saprotrophic fungi and bacteria (Baldrian, 2017). High soil biodiversity supports functional flexibility for the adaptation to unknown changes that may occur in the future.

In managed forests, the quantity of photosynthetic carbon allocated belowground to fine roots and root-associated microorganisms decreases (Noormets *et al.*, 2015). Therefore, changes in alpha and

beta diversity of root and soil microbiota are expected with increasing management intensity. We aim to achieve the intensity of forest management that still supports high soil biodiversity.

In the LIFE SySTEMiC project, ectomycorrhizal fungi actively associated with tree roots were identified using classical methods, including morpho-anatomical characterization and Sanger sequencing. The diversity and composition of rhizosphere and bulk soil microbiota was analysed with amplicon (NGS) sequencing.

The soil microbiome was studied in nine beech sites (Table 3.4.1) with different silvicultural approaches (see Table 3.1.1) in 2021. Samples were collected in October and November. In two of these sites (i.e. Site 02 - Baldo's forest and Site 03 - Pian dei Ciliegi), additional samples were included from a preliminary sampling in 2020 before harvesting the selected trees. The position of the cut trees in relation to the soil microbiome sampling trees is presented in Figure 3.4.1.

Table 3.4.1. List of beech sites where sampling for ectomycorrhiza and soil microbiome was performed.

Id	Site name	Bedrock
02	Baldo's forest	silicate
03	Pian dei Ciliegi	silicate
11	Fonte Novello	calcareous
12	Venacquaro	calcareous
14	Ogulin	calcareous
23	Osankarica	silicate
24	Pri Studencu	calcareous
25	Rajhenavski Rog	calcareous
29	Gorski kotar, Vrbovsko	calcareous

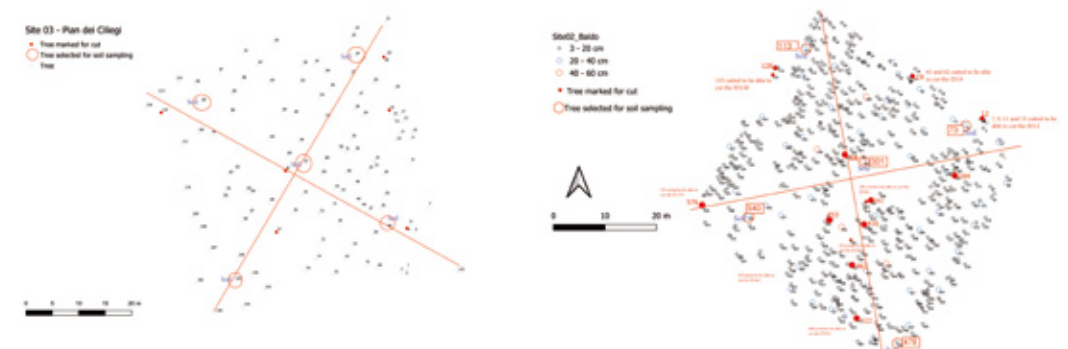


Figure 3.4.1. Position of cut trees in relation to the sampling points for soil biodiversity in Sites 2 and 3.

Sampling was performed at four points (N, S, E, W) at one-meter distance around each of the five selected trees per plot (for detailed description see <https://www.lifeyesystemic.eu>). At each of the four points, samples were collected separately for analyses of active ectomycorrhiza (EcM), NGS sequencing, and soil physic-chemical analyses. Sample preparation and analyses followed the protocols available on the LIFE SySTEMiC web page: <https://www.lifeyesystemic.eu/>. Data were rarified for both approaches to eliminate the effect of different sample sizes or sequencing depths.

3.4.1 Harvesting experiment

Based on the results from the amplicon sequencing, harvesting showed weak short-term effect on OTU (operational taxonomic unit) richness, Shannon diversity index or evenness of either all fungi or the subset of ectomycorrhizal fungi.

However, for the active EcM, the taxa (morphotype) richness and Shannon diversity index were significantly decreased after harvesting, while evenness showed no significant change (Figure 3.4.1.1).

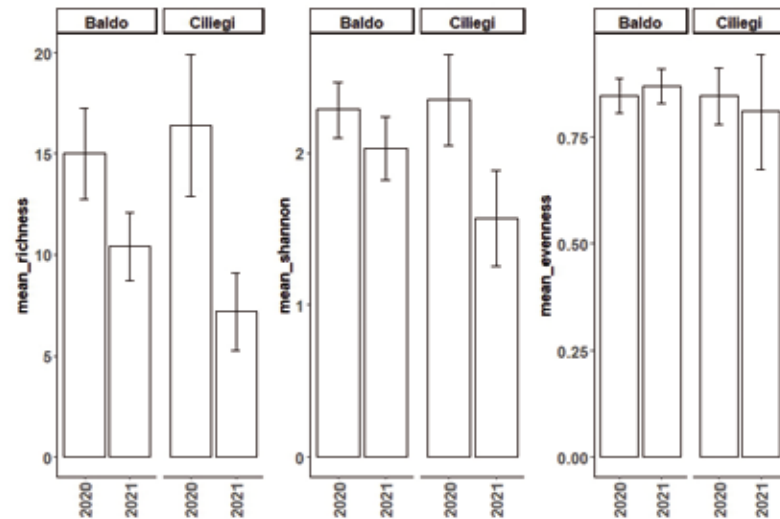


Figure 3.4.1.1. Pre - (2020) and post-harvesting (2021) values for alpha diversity (mean values for taxa richness, Shannon diversity index and Pielou's evenness) of active ectomycorrhizal fungi.

After harvesting, the total fungal community (Figure 3.4.1.2) was significantly different compared to the year before, the same was found for the subset of EcM fungi and active EcM. The effect of the site was significant, too.

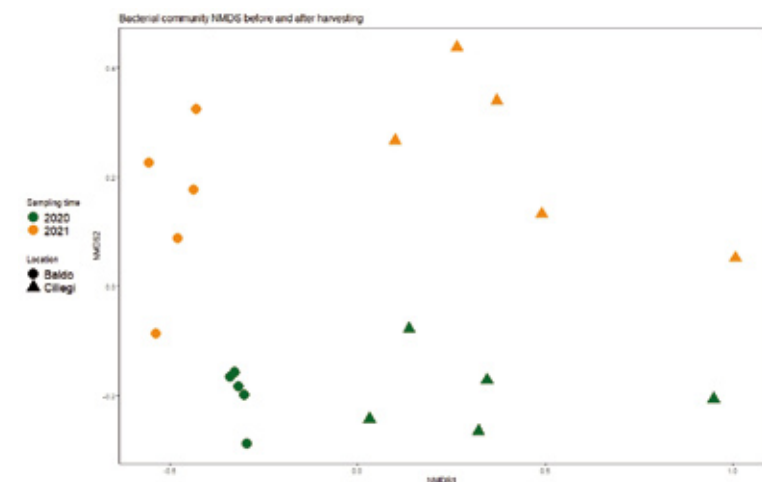


Figure 3.4.1.2. NMDS based on Bray-Curtis distance comparing total fungal communities before and after harvesting at two sites (Site 02 - Baldo's Forest and Site 3 - Pian dei Ciliegi), stress = 0.11.

After harvesting, the relative abundance of EcM OTUs decreased in both plots due to loss of EcM partners and reduced carbon flow belowground, while the relative abundance of saprotrophs increased because of increased necromass. Among ectomycorrhizal OTUs, the relative abundance of *Russula* OTUs decreased in 2021 relative to 2020 (pre-harvesting), while the relative abundance of *Cortinarius* OTUs increased in both sites.

3.4.2 Long-term effect of silviculture

The highest levels of OTU richness for the total fungal community (Fig. 3.4.2.1) were detected in unmanaged Site 11 - Fonte Novello and Site 25 - Rajhenavski Rog along with Site 29 - Gorski kotar, Vrbovsko which is managed according to the individual tree selection system. However, the amount of total deadwood is around five times the amount of deadwood in Site 02 - Baldo's forest which is managed in the same way (Table 3.2.2).

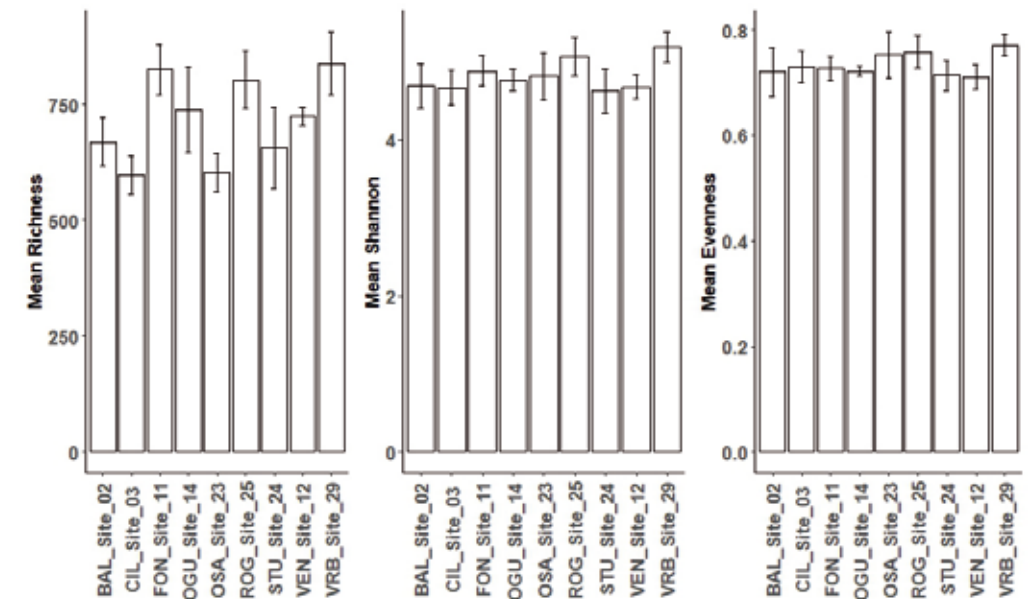


Figure 3.4.2.1. Comparison of alpha diversity indices of all fungi at beech forest sites.

The subset of EcM fungi (active EcM along with the dormant propagules) (Figure 3.4.2.2) showed the highest values for Site 14 - Ogulin and Site 29 - Gorski kotar, Vrbovsko which was consistent with the data for active EcM at these two sites (see Figure 3.4.2.3), but not with the data for Site 02 - Baldo's forest. Which active EcM develop in a certain time may depend on many environmental parameters, such as weather conditions, tree phenology etc.

The highest taxa richness and diversity of active EcM were recorded for the Site 02 - Baldo's forest, Site 14 - Ogulin and Site 29 - Gorski kotar, Vrbovsko (Figure 3.4.2.3). Site 02 - Baldo's forest and Site 29 - Gorski kotar, Vrbovsko are sites with the individual tree selection silvicultural systems, while the stand where Site 14 is located has a uniform shelterwood system. However, Site 14 itself has served as a seed forest object for the last ten years, and only individual dead trees and trees in poor health have been removed.

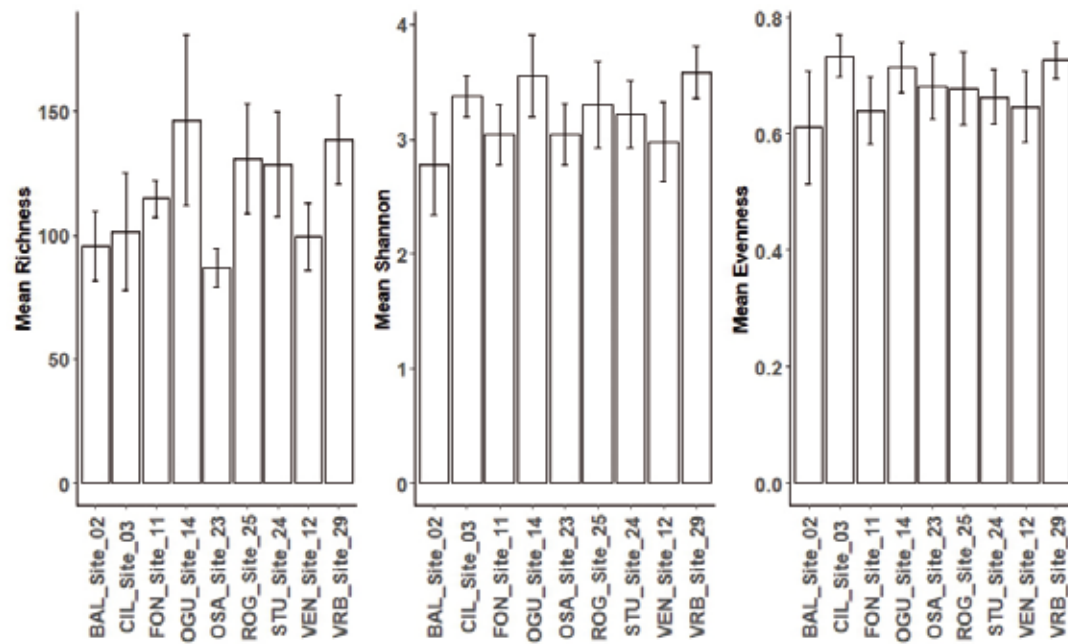


Figure 3.4.2.2. Comparison of alpha diversity indices for the subset of ectomycorrhizal fungi at beech forest sites.

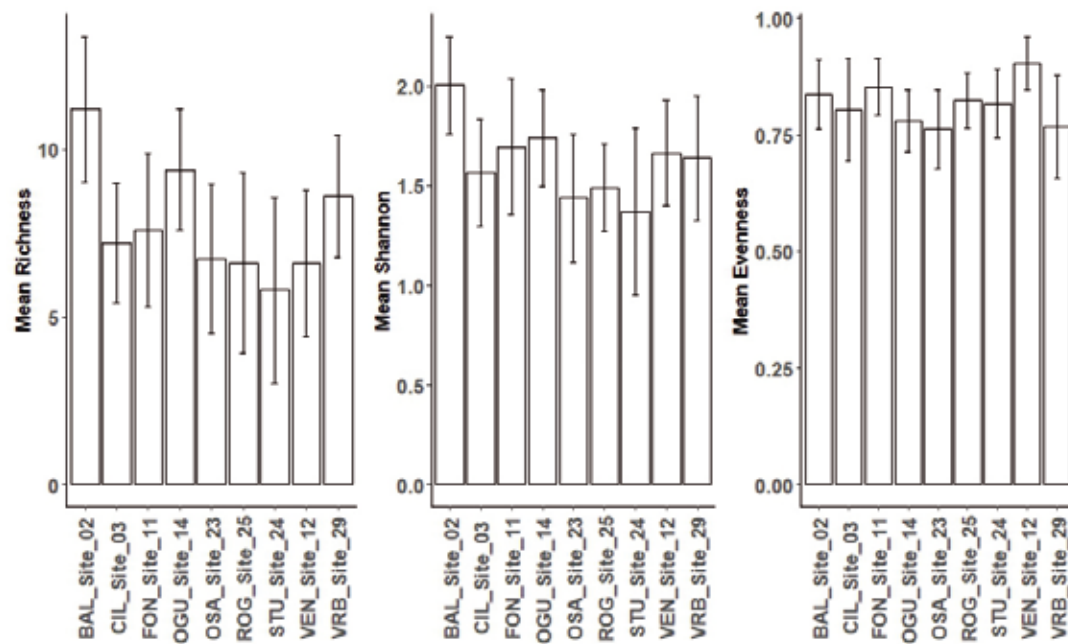


Figure 3.4.2.3. Alpha diversity indices (calculated from rarefied data) of active ectomycorrhiza for the investigated beech forest sites.

Silvicultural systems significantly influenced the richness of all fungi, with unmanaged and individual tree selection systems supporting higher values compared to uniform and irregular shelterwood systems (Figure 3.4.2.4a). This suggests that less intensive management practices are beneficial for fungal diversity. However, the effects on Shannon diversity were less pronounced, and there was no significant impact on evenness, indicating that the relative abundance distribution of species remains consistent across different management practices. In the EcM subset, silvicultural systems also significantly affected richness, mirroring the patterns observed in the total fungal community. Unmanaged and individual tree selection systems exhibited higher EcM richness, underscoring the importance of less intensive management for maintaining EcM diversity. The effects on Shannon diversity were significant but did not show clear distinctions among different management practices, similar to the total fungal community. Again, evenness was not significantly impacted.

Taxa richness of active EcM for the unmanaged sites was in the range of sites with shelterwood systems, while individual tree selection systems somewhat deviated into positive direction (Figure 3.4.2.4b). High levels of taxa richness for active EcM in the individual tree selection system could be related to the higher complexity of forest structure in combination with removal of individual canopy trees that prevents transition into the climax state of succession. According to literature (Twieg *et al.*, 2007) the highest species richness of ectomycorrhizal fungi occurs at canopy closure, which is characterized by the highest tree growth rates and maximal leaf area which corresponds to higher allocation of carbon belowground, to roots and root symbionts (Twieg *et al.*, 2007). The effects on Shannon diversity showed no clear patterns of differences among the management practices, and there was no significant impact on evenness.

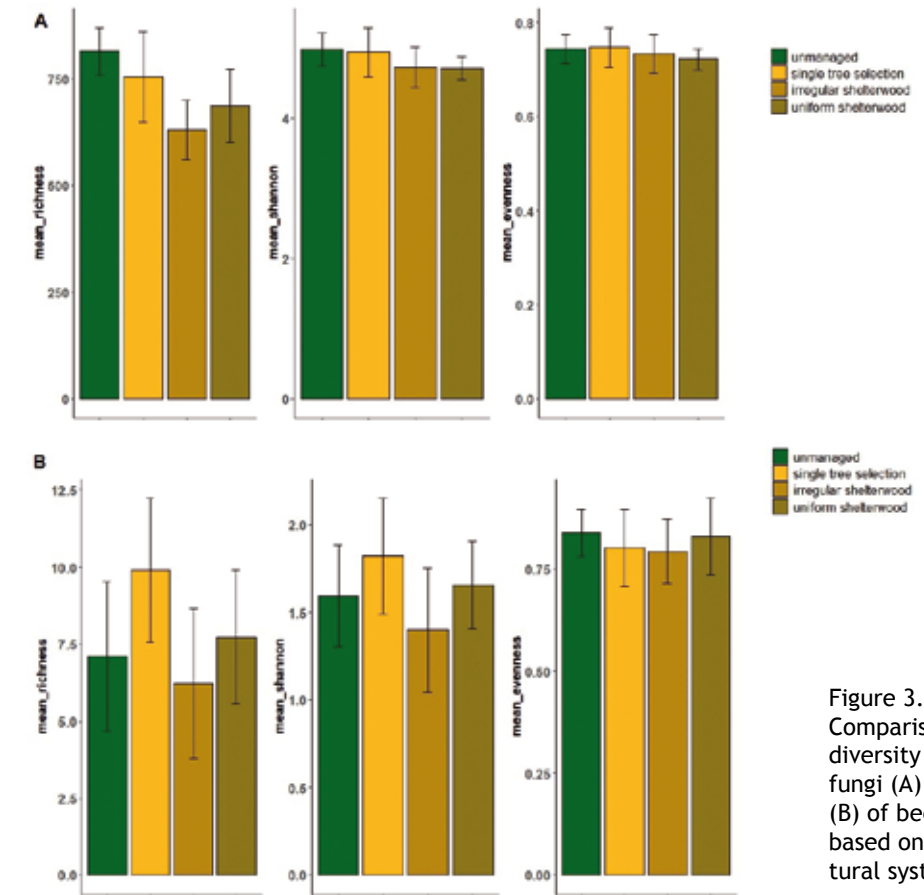


Figure 3.4.2.4. Comparison of alpha diversity indices of all fungi (A) and active EcM (B) of beech forest sites based on the silvicultural system.

While silvicultural systems do have an impact on fungal communities, their effect is often overshadowed by stronger influences of location and bedrock when accounted for. The significant effects of these variables suggest that local environmental conditions and regional differences are crucial determinants of fungal diversity and community structure. Consequently, while management practices are important, they should be considered alongside these other influential factors for effective conservation and management of fungal biodiversity.

When examining beta diversity, which reflects changes in fungal community composition across different sites, the analysis revealed that bedrock type plays a critical role in shaping both the total fungal community and the EcM community (Figure 3.4.2.5).

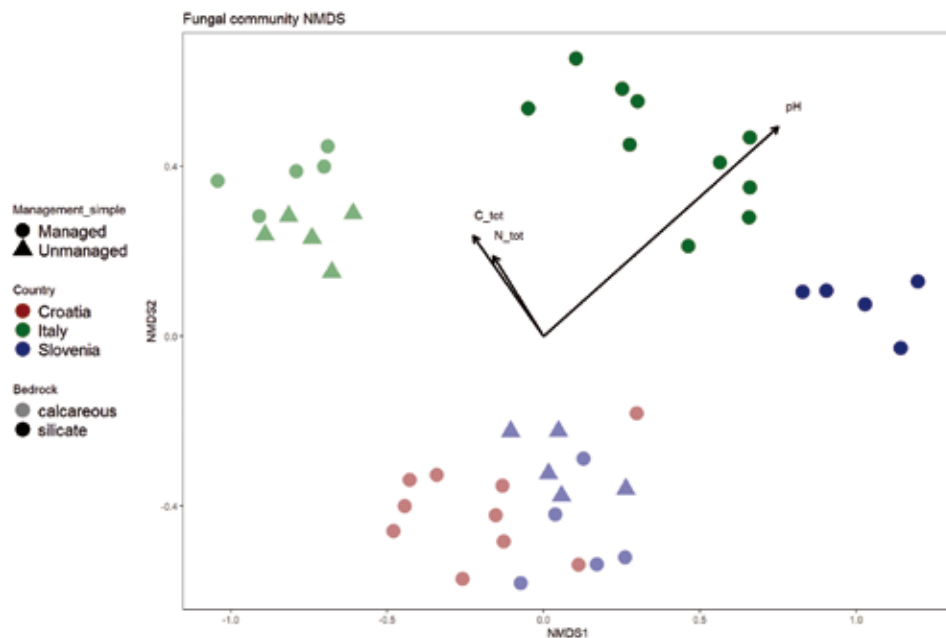


Figure 3.4.2.5. NMDS based on Bray-Curtis distance (stress=0.1050) for communities of all fungi as affected by the bedrock, location and management (unmanaged vs. managed).

PERMANOVA results showed that bedrock significantly influenced the composition of these communities, suggesting that the underlying geology and soil chemistry are among key drivers of fungal community composition. This influence was more pronounced than that of silvicultural practices, highlighting the foundational impact of abiotic factors on fungal communities. For the active EcM community, while silvicultural practices did affect community composition, the effects of bedrock remained substantial, underscoring its primary influence.

3.5 GenBioSilvi model

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Measuring genetic diversity is crucial to understand the health status of forest ecosystems in response to climatic change as well as supporting conservation and management efforts.

Based on the results obtained, we can assume that there is high genetic diversity in all sites, but we can't conclude that there is significant genetic diversity between the sites even though they differ in management types. Analyzing the pattern of genetic diversity distribution based on SSRs data, we observed that old-growth forests have a complex and heterogeneous spatial genetic structure. The spatial genetic structures observed in old-growth beech forests can be attributed to the non-random mating between closely related individuals. Most beech seeds germinate close to mother trees, thus contributing to the formation of such family structures. The management can affect the population demography.

From the results obtained for each management type (see the deliverable Action B2 GenBioSilvi model), it was possible to observe a complex spatial genetic structure similar to that reported for old-growth forests at sites managed with individual tree selection thinning. Site 02 - Baldo's Forest, monospecific beech stands, reported the most complex spatial genetic structure among the managed sites. Observing the various stands managed according to the uniform and irregular shelterwood, we observed a trend of simplified spatial genetic structure. The complexity of the old-growth forest and Site 02 - Baldo's forest implies a dynamic and adaptable ecosystem capable of responding to environmental changes by generating new genetic variability through recombination and gene flow between subpopulations. In addition, we observed a high number of SNPs correlated with current environmental conditions across sites. The presence of these allelic variants associated with bioclimatic indicators that best characterize the local environment is important. We found that Site 02 - Baldo's forest presented a higher number of SNPs correlated with bioclimatic indicators and a complex spatial genetic structure. Based on our results, we assumed that better conditions are found in old-growth forests and individual tree selection system sites. Stand structure is a key element in assessing ecological functions and services in forest ecosystems.

To describe biodiversity, we observed dendrometric data to define the forest structure of the analyzed sites. Thanks to this data, we can assume that the best structure observed is that characteristic of a multi-layered uneven-aged forest. The sites that presented these characteristics are Site 02 - Baldo's Forest, Site 11 - Fonte Novello, and Site 25 - Rajhenavski Rog. A multi-layered uneven-aged population supports the presence of natural regeneration because it shows gaps in canopy cover that create ideal conditions for the establishment of natural regeneration. Furthermore, a multi-layered forest increases the probability of sporadic species occurrence. In addition, multi-layered forest allows high pollen dispersal, promoting genetic diversity. Therefore, based on the best parameters used to define forest structure, we observed that sites characterized by multilayered uneven-aged forest structure also exhibit complex spatial genetic structure and high genetic diversity.

Deadwood plays crucial functional roles such as in nutrient cycling and as a carbon sink. The presence of deadwood can promote germination and seedling development of tree species, acting as nursery sites and thus contributing to forest renewal.

Regarding the amount of deadwood, the best situation is observed in unmanaged sites that showed a higher volume of deadwood.

Deadwood provides an important microhabitat for the development and conservation of species important to the forest ecosystem. In addition to deadwood, we observed other indicators related to saproxylic microhabitats. The greatest number of microhabitats was detected at sites where there are century-old individuals with stem deformations and cavities. The frequency of epixylic microhabitats is lower than saproxylic in all observed populations. Epixylic are used as indicators to assess ecosystem conditions. The presence of these microhabitats is an important source of biodiversity. Based on the obtained results, management types that allow for complex forest structures characteristic of uneven-aged and multilayered stands increase the probability of observing different microhabitat forms.

The majority of analyzed sites are pure beech stands. Some sites such as Site 29 - Gorski kotar, Vrbovsko, Site 25 - Rajhenavski Rog, and Site 12 - Venacquaro present sporadic species such as *Abies alba* Mill., *Prunus avium* L., *Acer* spp. L., and *Fraxinus* spp. L. Sporadic species should be maintained and where they exhibit natural regeneration this should be preserved and conserved and/or stimulated.

Forest management practices, especially those involving tree harvesting, influence the soil community, pivotal components for nutrient cycling, and the well-being of trees. These practices alter sub-soil microbial dynamics, potentially leading to a reduction in ectomycorrhizal fungal diversity. This decline can hamper tree growth and compromise the overall functionality of forest ecosystems. Consequently, understanding these effects is imperative for fostering sustainable forest management.

The marked and significant differences observed in bacterial, ectomycorrhizal, and fungal community compositions between close-to-nature, unmanaged, and medium intensity management strategies are attributable to a variety of factors. Each management approach represents a distinct level of disturbance to the forest ecosystem. Close-to-nature management aims to emulate natural disturbances with minimal human interference, while unmanaged forests experience no human intervention. Medium intensity management, however, may involve more intensive silviculture systems, thereby introducing greater disruption to the ecosystem. In conclusion, we assumed that better soil diversity is present in less impact and in old-growth forest-managed sites.

Analysing results, we observed that the condition that conserves and/or increases diversity is the managed site with individual tree selection. This type of management is less impactful moreover it mimics conditions normally found in old-growth forests and unmanaged forests. The removal of a few individuals based on phenotype and position results in the formation of gaps in the tree cover that encourages the growth of natural regeneration. Compared to old-growth forests, where regeneration is predominantly on the borders, in sites managed with individual tree system, natural regeneration is also stimulated within the stand. This type of management increases the complexity of the forest structure presenting a multi-layered pattern, a typical characteristic of an uneven-aged forest. This allows better pollen dispersal -high gene flow- since fewer barriers are present. This promotes genetic diversity and increases spatial genetic complexity, leading to a greater occurrence probability of new allelic variants that are essential in responding to climate change. In addition, a key aspect of forest stand resilience is the presence of high biodiversity. Through our results, we have observed that stands that exhibit higher genetic diversity also exhibits higher biodiversity at the ecosystem and species levels.

Based on the results obtained during the analyses, through the observation of these indicators it is possible to simulate the genetic diversity status of the stand and guide users toward sustainable management to conserve and/or increase the genetic diversity present.

Considering the results obtained from the analysis of all biodiversity indicators, we developed a model that describes the current status of genetic resources within the site. This model was developed to help forest users for checking the current status of stand biodiversity and providing guidelines for sustainable management. To develop a user-friendly suitable model, we observed that it is possible to identify a set of key indicators that are more representative. The indicators at the basis of the model can indirectly describe the genetic diversity status of the stand, as seen from the results obtained. In addition, it is also possible to identify some indicators that are more representative of biodiversity in terms of deadwood, microhabitat, and species diversity. For this reason, we decided to exclude in the form developed for users the data collection on genetic diversity and soil diversity that are difficult to observe. In this regard, we have developed a model that involves the observation of some key indicators that describe the current status of the analyzed stand. The selected indicators are shown in Table 3.5.1.

Table 3.5.1. Description of selected indicator useful for users to describe the status of the stand.

Categories	Indicators	Description
Forest structure	DBH standard deviation	Variability in tree DBH within the stand
	Percentage of trees in regeneration layer	Presence of the target species natural regeneration
	DBH class distribution curve	Complexity of horizontal and vertical forest structure
Deadwood	No. of population strata	
	Standing deadwood presence	Description the presence of deadwood functioning as microhabitat
	Coarse woody debris	
Species diversity	Species richness	Number of all species present in the stand regarding both the presence of adults' individuals and regeneration
	Percentage of non-target individuals regeneration	
Microhabitat	Percentage of individuals with cavities	Presence of key microhabitat form for biodiversity
	Percentage of individuals with Injuries and wounds	
	Percentage of individuals with Deformation	

The model involved the production of a form that requires the compilation of certain parameters that can be easily observed in the field by users. Based on our results, we have chosen value thresholds for each selected indicator. The annotation of the actual real data for each indicator will produce a score based on the selected thresholds. The final score is associated with the identification of the current status of the stand analyzed. For each final score, indications are provided regarding possible actions to be taken to implement sustainable management of the stand (Figure 3.5.1). Below is the example of the form we produced for compilation (Figure 3.5.2). Instead, Figure 3.5.3 shows the compiled form based on the actual data obtained from the Site 02 - Baldo's forest.

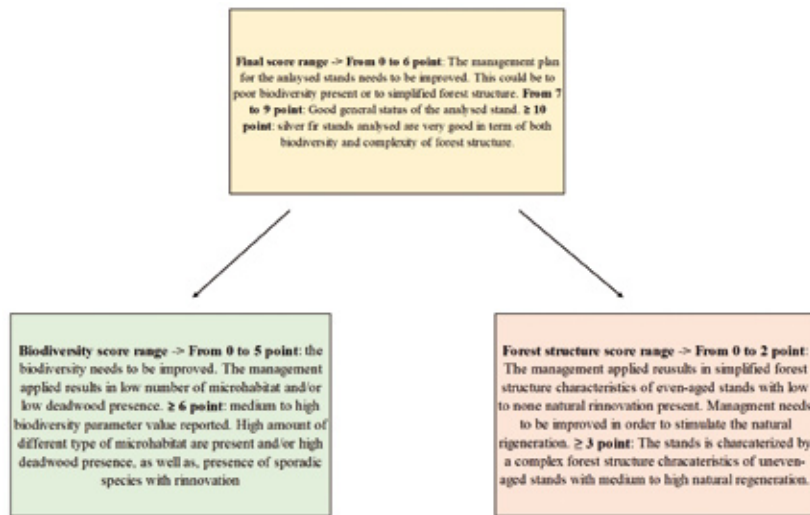


Figure 3.5.1. Guideline for forest management based on the final score obtained after forest assessment forest form compilation.

Forest stand evaluation form					
Forest management	Real Data		Implication for Management		
Forests should be managed in a way to preserve their multifunctional role (ecological, social and productive forest functions). This can be achieved only through maintenance of healthy forests and their biodiversity, protection of its natural fertility and water sources as well as other beneficial functions of forests in the water and carbon cycle, sustainable supply of wood and other products from forest, profit and employment.					
Forest structure indicator	Real Data	Indicator-specific score	Implication for Horizontal and Vertical Forest structure		
Forest structure is both a product and driver of ecosystem processes and biological diversity. Changes in forest structure as a result of management for timber production have undesirable consequences for other components of forest ecosystems	Structural indicator	<input checked="" type="checkbox"/> Score 1 (DBH SD < 5)	Results from annotated parameters		
		<input type="checkbox"/> Score 2 (5 ≤ DBH SD ≤ 15)			
	<input type="checkbox"/> Score 3 (DBH SD > 15)	<input type="checkbox"/> Score 1 (Single forest structure without regeneration) - From 4 to 6			
	Refers to % of trees in regeneration layer (considering DBH less than 10 cm)	<input type="checkbox"/> Score 1 (% Rim < 15)		<input type="checkbox"/> Score 2 (Simplified forest structure with regeneration or Complex forest structure without regeneration) - From 7 to 8	
		<input type="checkbox"/> Score 2 (15 ≤ % Rim < 30)		<input type="checkbox"/> Score 3 (Complex forest structure with regeneration) - From 9 to 11	
<input type="checkbox"/> Score 3 (30 ≤ % Rim < 50)		<input type="checkbox"/> Score 4 (Complex forest structure with regeneration present in the total area) - From 12 to 13			
Curve of DBH class distribution	<input type="checkbox"/> Score 1 (Bell-shaped)				
N° of strata (stratification of population)	<input type="checkbox"/> Score 2 (Multi-modal)				
	<input type="checkbox"/> Score 3 (J-shaped)				
	<input type="checkbox"/> Score 4 (Mono-modal)				
Productivity indicators	Real data	Indicator-specific score	Implication for population biodiversity		
				<input type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%)	
				<input type="checkbox"/> Score 3 (% Ind. > 50%)	
Forest site productivity is the production that can be realized at a certain site with a given genotype and a specified management regime. Site productivity depends both on natural factors inherent to the site and on management-related factors.	Growing stock (m³/ha)	In managed stands, the minimum growing stock should be around 300-350 m³/ha. This might be applicable for shade tolerant species			
		Biodiversity indicators	Real data	Indicator-specific score	Implication for stand biodiversity
<input type="checkbox"/> Score 2 (Presence of standing deadwood)	<input type="checkbox"/> Score 1 (Total absence of deadwood) - 2 point				

Biodiversity conservation is a key objective for sustainable forest management, but the multi-dimensional and multi-scale character of biodiversity renders full assessment difficult at large scale. Therefore, indicators are often used to monitor biodiversity. The quantity and quality of deadwood are commonly used in nature conservation as indicators of forest biodiversity. Additionally, the presence of species different from the target species as well as the presence of specific type of microhabitat are also used to monitor biodiversity in forest stand.	Course woody debris	<input type="checkbox"/> Score 1 (Absence of Course woody debris)	<input type="checkbox"/> Score 2 (Presence of standing deadwood Course woody debris) - 3 point	
		<input type="checkbox"/> Score 2 (Presence of Course woody debris)	<input type="checkbox"/> Score 3 (Presence of various type of deadwood) - 4 point	
	Species diversity indicators	Indicator-specific score		Implication for stand biodiversity
	Species richness (presence of individual of non-target species)	<input type="checkbox"/> Score 1 (Absence of other species)	Results from annotated parameters	
		<input type="checkbox"/> Score 2 (Presence of other species)		
	% of non-target individuals in the smallest diameter class (10 cm)	<input checked="" type="checkbox"/> Score 1 (% Rim < 15)	<input type="checkbox"/> Score 2 (Sporadic species with some or low regeneration) - 3 point	
		<input type="checkbox"/> Score 2 (15 ≤ % Rim < 30)		
		<input type="checkbox"/> Score 3 (30 ≤ % Rim < 50)		<input type="checkbox"/> Score 3 (Sporadic species with high regeneration) - 4 to 6 point
		<input type="checkbox"/> Score 4 (% Rim > 50)		
	Microhabitat indicators	Real data	Indicator-specific score	Implication for stand biodiversity
% of individuals with cavities	<input checked="" type="checkbox"/> Score 1 (% Ind. ≤ 10%)	Results from annotated parameters		
	<input type="checkbox"/> Score 2 (10% < % Ind. ≤ 50%)			
% of individuals with injuries and wound	<input checked="" type="checkbox"/> Score 1 (% Ind. ≤ 10%)	<input checked="" type="checkbox"/> Score 1 (Absence of low number of microhabitat) - 3 point		
	<input type="checkbox"/> Score 2 (10% < % Ind. ≤ 50%)			
% of individuals with deformation	<input type="checkbox"/> Score 3 (% Ind. > 50%)	<input type="checkbox"/> Score 2 (Sporadic to consistent presence of microhabitat) - 4 to 6 point		
	<input type="checkbox"/> Score 2 (10% < % Ind. ≤ 50%)			
			<input type="checkbox"/> Score 3 (High presence of microhabitat) - 7 to 9 point	
Final Score: 1				

Figure 3.5.2. Forest population assessment form structure.



Forest population assessment sheet - Simulation Site 02 (Baldo's forest)							
Forest management	Real Data		Implication for Management				
Forests should be managed in a way to preserve their multifunctional role (ecological, social and productive forest functions). This can be achieved only through maintenance of healthy forests and their biodiversity, protection of its natural fertility and water sources as well as other beneficial functions of forests in the water and carbon cycle, sustainable supply of wood and other products from forest, profit and employment.	Single tree selection thinning						
Forest structure indicator	Real Data	Indicator-specific score		Implication for Horizontal and Vertical Forest structure			
Forest structure is both a product and driver of ecosystem processes and biological diversity. Changes in forest structure as a result of management for timber production have undesirable consequences for other components of forest ecosystems	DBH Standar deviation	9,55	<input type="checkbox"/> Score 1 (DBH SD < 5)	Results from annotated parameters			
			<input checked="" type="checkbox"/> Score 2 (5 ≤ DBH SD ≤ 15)				
			<input type="checkbox"/> Score 3 (DBH SD > 15)				
	Refers to % of trees in regeneration layer (considering DBH less than 10 cm)	83,54	<input type="checkbox"/> Score 1 (% Rim < 15)				
			<input type="checkbox"/> Score 2 (15 ≤ % Rim < 30)				
Curve of DBH class distribution	J-shaped	<input type="checkbox"/> Score 3 (30 ≤ % Rim < 50)					
		<input checked="" type="checkbox"/> Score 4 (% Rim ≥ 50)					
N° of strata (stratification of population)	Tri-stratified	<input type="checkbox"/> Score 1 (Bell-shaped)	<input type="checkbox"/> Score 1 (Simple forest structure without rinnovation) - From 4 to 6				
		<input type="checkbox"/> Score 2 (Multi-modal)					
		<input checked="" type="checkbox"/> Score 3 (J-shaped)					
Productivity indicators		Real data		Implication for population biodiversity			
Forest site productivity is the production that can be realized at a certain site with a given genotype and a specified management regime. Site productivity depends both on natural factors inherent to the site and on management-related factors.	Growing stock (m³/ha)	305 m³/ha	In managed stands, the minimum growing stock should be around 300-350 m³/ha. This might be applicable for shade tolerant species				
Biodiversity indicators	Real data	Indicator-specific score		Implication for stand biodiversity			
Biodiversity conservation is a key objective for sustainable forest management, but the multi-dimensional and multi-scale character of biodiversity renders full assessment difficult at large scale. Therefore, indicators are often used to monitor biodiversity. The quantity and quality of deadwood are commonly used in nature conservation as indicators of forest biodiversity. Additionally, the presence of species different from the target species as well as the presence of specific type of microhabitat are also used to monitor biodiversity in forest stand.	Deadwood indicators	Presence	<input type="checkbox"/> Score 1 (Absence of standing deadwood)	Results from annotated parameters			
			<input checked="" type="checkbox"/> Score 2 (Presence of standing deadwood)				
	Coarse woody debris	Absence	<input checked="" type="checkbox"/> Score 1 (Absence of Coarse woody debris)	<input checked="" type="checkbox"/> Score 2 (Presence of standing deadwood Coarse woody debris) - 3 point			
			<input type="checkbox"/> Score 2 (Presence of Coarse woody debris)				
Species diversity indicators	Real data	Absence	<input checked="" type="checkbox"/> Score 1 (Absence of other species)	Results from annotated parameters			
			<input type="checkbox"/> Score 2 (Presence of other species)				
			<input checked="" type="checkbox"/> Score 1 (% Rim < 15)		<input type="checkbox"/> Score 2 (Sporadic species with none or low regeneration) - 3 point		
			<input type="checkbox"/> Score 2 (15 ≤ % Rim < 30)				
% of non-target individuals in the smallest diametric class (10 cm)	0	<input type="checkbox"/> Score 3 (30 ≤ % Rim < 50)	<input type="checkbox"/> Score 3 (Sporadic species with high regeneration) - 4 to 6 point				
		<input type="checkbox"/> Score 4 (% Rim ≥ 50)					
Microhabitat indicators	Real data	14,51	<input checked="" type="checkbox"/> Score 1 (% Ind ≤ 15%)	Results from annotated parameters			
			<input type="checkbox"/> Score 2 (15% < % Ind ≤ 50%)				
			<input type="checkbox"/> Score 3 (% Ind > 50%)				
			% of individuals with injuries and wound		1,57	<input checked="" type="checkbox"/> Score 1 (% Ind ≤ 15%)	<input checked="" type="checkbox"/> Score 1 (Absence of low number of microhabitat) - 3 point
						<input type="checkbox"/> Score 2 (15% < % Ind ≤ 50%)	
% of individuals with deformation	7,57	<input type="checkbox"/> Score 3 (% Ind > 50%)	<input type="checkbox"/> Score 2 (Sporadic to consistent presence of microhabitat) - 4 to 6 point				
		<input type="checkbox"/> Score 1 (% Ind ≤ 15%)					
				Final Score: 8			

Figure 3.5.3. Forest population assessment form structure with Site 02-Baldo's forest data.

3.6 Recommendations for Sustainable Forest Management

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Overview of silvicultural systems

Sustainable and close to nature forestry uses forest management methods that promote conservation of nature and forests in such a way that they are preserved as natural ecosystems with all their diverse life forms and the relationships that exist within them. It is based on detailed forest management planning, adapted to individual site and stand conditions as well as forest functions, and considering natural processes and structures specific to natural forest ecosystems. Natural processes are altered as little as possible, while still maintaining the financial profitability and social sustainability of forest management (Veselič, 2008).

Silvicultural systems for beech stands should be carefully selected in order to promote close-to-nature approaches and mimic natural processes in forest stands. Within LIFE SySTEMiC four silvicultural approaches in beech stands were analysed:

- Irregular shelterwood system where forest regeneration takes place in openings and gaps,
- individual tree selection system where trees of different dimensions and age are presented on a small surface area,
- uniform shelterwood where the old stand is removed in a series of cuttings (usually three cuttings in case of beech) for establishment of a new even-aged stand under the shelter of the old one,
- Unmanaged forests, where natural processes in forest are not affected by forest management.

Analysed silvicultural systems are described in detail in the introduction of this handbook.

The LIFE SySTEMiC project analysed twelve beech stands, corresponding to four European Forest Types (EFT) has been studied: 6.6 Illyrian sub-mountainous beech forest; 7.2 Central European mountainous beech forest; 7.3 Apennine-Corsican mountainous beech forest and 7.4 Illyrian mountainous beech forest. Results, described in previous chapters, show that silvicultural systems with reduced impact are more favorable in light of genetic variability preservation, such as individual tree selection and low scale irregular shelterwood system.



Figure 3.6.1. Irregular shelterwood system is a common way for management of beech stands.

Silvicultural characteristics of beech

Beech is a shade tolerant tree species which is present in many forest plant communities in Europe and different forest types, mostly as dominant or in some cases as affiliated tree species. It is characterized by slow growth in youth, which culminates only after 45 years when light conditions allow that. Beech volume increment reaches its maximum very lately, on some sites only after the age of 75 years. In general, the average total volume increment does not show signs of decline up to the age of 150 years. Beech also responds to silvicultural measures or thinning until old age and surpasses all the other tree species (Diaci, 2006).

Threats

The beech is relatively resistant to most diseases. It does not suffer from massive pest infestations that lead to the total death of stands. Late spring frosts often damage young trees or flowers, which emerge simultaneously with leaf flush. Intense sunlight may damage the stem surface. Aphids may attack the bark and the fungus *Nectria ditissima* causes bark necrosis (Wuehlisch, 2010)

Main threats to beech forests due to climate change are natural disasters such as sleet and snowbreak at higher altitudes, as well as wind can cause major damage in these forests. Beech forest on warmer sites can be at risk due to stronger summer droughts. Forests on northern slopes are less threatened (ZGS, 2021).

Regarding biotic factors it is considered that these forests can be damaged by indigenous harmful organisms in combination with the weakening of beech vitality due to climate warming (complex causes of drying, phytophthora) and possible invasions of new diseases and pests.

The stability of beech stands with a higher proportion of other species is strongly threatened by health status of those species (for example spruce and gradations of bark beetle). There is also an increased risk of introduction and spread of invasive non-native species in forests near settlements and infrastructure facilities (roads, railways). An important threat to young trees is also represented by wild game browsing (ZGS, 2021).



Figure 3.6.2. Sleet can be very harmful for beech stands on higher altitudes.

Assessment of adaptation potential of beech to climate change

The assessment of the adaptation potential of beech forests to climate change shows that due to the successful rejuvenation and relatively high adaptability of beech to changes in the environment, the adaptability of the beech forests is quite high. The adaptation potential in the future depends mainly on active low intensity management, on the degree of silvicultural interventions and reduction of the game browsing damages (ZGS, 2021).

Regeneration of beech stands

Close-to-nature forestry mimics natural processes and structures as far as possible. Through natural regeneration of beech forest stands, trees' adaptability to conditions of specific growing sites and natural dynamics is preserved. Beech forest stands should be renewed naturally and should imitate a mixture of tree species and forest stands of natural forests (ZGS, 2021).

The natural regeneration of beech stands is generally not problematic. Young beech stands are characterized dense and vigorous layering. In gaps and uneven young stands dominant trees quickly develop overgrown crowns (Diaci, 2006).

The common way of regenerating beech stands is under canopy cover. Regeneration is set in the sequence of feelings, mostly as uniform shelterwood silvicultural system (Nocentini, 2009) or as a part of irregular shelterwood system. Irregular shelterwood is planned in small rejuvenation gaps (one to two tree height) which are then gradually connected with each other with time. A very suitable system is also a individual tree selection, especially in mixed stands with silver fir and on areas, where the pressure of a climatically unstable environment is very high (ZGS, 2021). On the other hand, regeneration can also be introduced on slightly larger areas (over 2 ha), which should be spatially separated by mature stands. This silvicultural approach has already some characteristics of uniform shelterwood forest management.

Regeneration in gaps should start after the year of a full seed mast with a felling intensity between 30 and 50% of the wood growing stock (Matić *et al.* 2003). It is necessary to prepare the stand for natural regeneration by cutting the understory trees and the shrub layer. Where possible, natural regeneration is used, as this makes the evolutionary process less disrupted and preserves the genetic diversity of forest tree populations. After the appearance of high-quality saplings, restoration is continued with a higher intensity of felling (between 50 and 60% of the wood stock) in order to increase the competitiveness of other important species, which are the part of beech forest communities. We complete the restoration with the last cuts at the latest when the young trees are 1 to 2 metres high. All regeneration feelings on areas with already established young stands have to be carried out outside the vegetation period (ZGS 2021).

In regeneration areas where natural regeneration fails it is recommended to restore the forest by planting and enrichment planting of beech seedlings with admixture of other site suitable tree species (such as oaks, sycamore maple, other noble hardwood species). The enrichment planting should be carried out only in small groups of trees. The size of the areas for restoration should be as small as possible - the mosaic structure of future stands is increasing their resilience to threats of climate change.

Despite the fact that natural restoration is a priority choice, regeneration by planting and sowing should be carefully considered. The disadvantage of natural regeneration is that forest resources can be endangered, since long-lived tree species cannot adapt to new diseases and pests quickly enough simply by transferring genetic information through natural evolutionary processes. Particularly problematic are damaged areas where trees have damaged regenerative parts of their crowns, which are supposed to ensure natural rejuvenation (ZGS 2021).

Tending and protection of beech stands

Most suitable tending and protection measures are summarized on the basis of SFM guidelines in Slovenia (ZGS, 2021) and LIFE SySTEMiC results. From a silvicultural point of view, beech, at a suitable density, quickly clears its branches so thinning measures at young stages mostly include removal of poorly formed, damaged and pre-grown trees. Trees in understorey shouldn't be removed since they are an important part of stability of future stand. By regulating the tree composition, we have to maintain tree species biodiversity and reduce the proportion of unwanted tree species.

Special attention should be paid to the stability of the beech stands, especially on slopes. With new knowledge about the development of forest stands, due to the growing economic importance of forest management and due to the increasing need for greater resistance of stands, updates and adaptation of silvicultural measures are needed. In a period of increasing frequency and severity of natural disasters, the issues of stability and resilience of beech stands are becoming increasingly important. With intensive silvicultural treatment of beech stands its stability is threatened. So called situational thinning, which takes into account the classic silvicultural principles, but focuses on a small number of selected target trees is a more suitable solution. Parts of the forest stand can be left to natural development and natural automaticity which also preserves its genetic diversity (Sever *et al.* 2022).

When choosing target trees, the principle applies that tree vitality and stability take priority over quality, which in turn takes precedence over the distance between target trees. The intensity of the removal of competing trees for individual target trees is adjusted to the conditions in the forest stand and tree species (Diaci, 2021).

A gradual natural transformation (restoration) of adult beech stands is necessary only where tree species composition is changed to the extent that endangers its stability. The change should go in the direction of natural renewal with deciduous trees, especially beech and all noble deciduous trees including cherry and linden.

Forest protection measures in beech stands are directed into preventive measures such as shortening of production periods in most vulnerable stands, improvement of stability of stands - timely and sufficient thinnings, which will ensure an appropriate ratio between the diameter and the height of the tree, the tree crowns must have enough growth space, maintaining of a structured forest edge and protection of natural young growth from excessive game browsing (ZGS, 2021).



Figure 3.6.3. Thinning is still a part of beech stands management in their adult age.

Adaptation of beech stands to climate change

The main measures to adapt forest management of beech stands to climate change are focusing on adaptation of tree composition in beech forest stands, increase of forest resilience by diverse structures of forest stands on all levels, especially genetic, through advanced forest regeneration and reforestation measures, and increase of their stability by early enough tending measures (e.g. thinning), formation of multilayered and selective forest structures in suitable stands, and (last but not least) monitoring and conservation of forest biodiversity, starting at genetic diversity (Bajc *et al.*, 2020).

Forest restoration of beech stands offers the possibility of choosing tree species and their provenances, which we assume are better adapted or adaptable to the changing environment. On the other hand, during the period of restoration, the forest is particularly sensitive to changes in the climate, as young trees are especially sensitive to drought and other extreme climatic conditions. Selection pressure on the young growth improves the chances of survival of adult stands that develop from the young stages of forest stand development. It is necessary to choose beech provenances and tree species mixtures that are adapted to different growing conditions, which can form stable and structurally diverse forest stands.

A key measure for adapting forest management will also be increasing the stability and resistance of beech stands to the expected more frequent natural hazards (icebreaks, snowbreaks). Adequate solutions are found in the mosaic structuring of forest stands, in stand structures close to individual tree selection system and in timely thinning of young and middle age stands. The stability of stand structures can also be increased by reducing the size of standing gaps, formation of forest edges, shortening of production periods and admixture of tree species with deep root systems (Breznikar, 2019).

In order to ensure the adaptability of future stands to changes in the environment, it is necessary to use genetically diverse forest reproductive material (FRM), by obtaining seeds from a large number of trees during the period of strong flowering and fruiting, professionally appropriate mixing of the FRM, and, on the basis of provenance tests, scientifically grounded transfer of different local provenances between different ecological environments. In addition, appropriate medium-term planning of the storage of seed stocks in the seed bank and the constant availability of appropriate seedlings are important (Bajc *et al.*, 2020).



Figure 3.6.4. Successful regeneration of beech stands with planting depends on quality and genetically diverse seed sources.



4. SUSTAINABLE FOREST MANAGEMENT OF *PINUS* spp. (*Pinus nigra* J.F. Arnold, *Pinus pinea* L., *Pinus pinaster* Aiton)

4.1 Introduction

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Pinus spp.

Pines in Europe are among the most significant ecological and economic tree genera in the Mediterranean and sub-Mediterranean regions. Globally, they are one of the largest genera taxonomically, with over one hundred species primarily growing in the Northern Hemisphere. Pines inhabit well-lit areas and are adaptable to various ecological conditions, including temperature, moisture, and soil. They can live up to 250 years. Pines are evergreen conifers with characteristic needles, which can be in pairs (two-needled), in threes (three-needled), or in fives (five-needled). Their cones (fruit) mature in the second or third year.

Pinus nigra L. - Biology and area distribution

Black pine (*Pinus nigra* J.F. Arnold) is a evergreen conifer native to the Mediterranean region and parts of Europe. It can be subdivided into five subspecies based on geographical distribution, needle length, and needle stiffness: *P. nigra* J.F. Arnold subsp. *nigra*, distributed in southeastern Austria, northern Italy, the Balkan Peninsula, Bulgaria, Romania, Turkey-in Europe; *P. nigra* subsp. *Salzmannii* (Dunal) Franco, distributed in southwest Europe, France (Hérault, Pyrenees), Spain, Algeria and Morocco, *P. nigra* subsp. *larico* (Poir.) Palib. Ex Maire, distributed in France and Italy; *P. nigra* subsp. *dalmatica* (Vis.) Franco, distributed in Croatia; *P. nigra* subsp. *pallasiana* (Lamb.) Holmboe, distributed in Greece, Cyprus, southwest Bulgaria, southeast North Macedonia, south Albania, and from Crimea along the Black Sea coast to Turkey (Gausson *et al.*, 1993; Farjon, 2017). The species grows in association with *Pinus sylvestris* L., *Pinus mugo* Turra, *Pinus halepensis* Mill., *Pinus pinea* L. and *Pinus haldreichii* Christ (Burns and Honkala, 1990).

Black pine typically forms pure stands but can also be found in mixed stands together with other pines, especially with *Pinus sylvestris* (Isajev *et al.*, 2004). This medium-sized pine that can grow up to 30 meters (rarely 40-50 meters) tall. Young black pine trees are tall and slender in shape, becoming rounder as the tree gets older, in some cases even developing a flattened, umbrella-shaped crown (Isajev *et al.*, 2004.). The bark of young individuals is brownish-grey and scaly, cracking and fissuring as the trees mature. Western varieties exhibit pale plaques, while eastern subspecies have darker, almost black grooves. In older trees the fissures become very deep and the plaques are much bigger (Eckenwalder, 2009). Buds are ovoid, pointed and resinous. Needles, which are growing in pairs, are 8-15(19) cm long, 1-2 mm thick, straight or curved and finely serrated. The colour is green, from pale to deep according to provenance, and have on each of the two sides 12-24 rows of stomata. They persist on the tree for 3-4 (8) years (Willis *et al.*, 1998).

Black pine is a monoecious species. Reproductive maturity is reached at the 15-20 years of age. The male catkins are bright yellow, cylindrical, and short-stalked, while the female inflorescences are bright red to purple, small, and short-stalked or sessile. Pollination is anemophilous and occurs from May to June when the pollen is released in great quantity. After pollination the cones begin to develop slowly. The mature cones are sessile and horizontally spreading, 4-8(9) cm long and 2-4 cm wide. The cones ripen from September to November of the second growing season, turning from yellow-green to shiny yellow-brown to light brown. They open in the third year after pollination. Cones contain 30-40 seeds which are grey, 5-7 mm long, with a wing 19-26 mm long (Isajev *et al.*,

2004). The seed dispersal occurs from October till November of the second growing season. Mast seeding occurs every two to five years.

Black pine stands exist at altitudes ranging from 350 m to 2200 m, with optimal altitudinal range being between 800 and 1500 m (Praciak *et al.*, 2013). It can grow on a variety of soils, from podzolic sands to limestone, often dependent on region and climate (Farjon and Filer, 2013). Black pine can grow in both extremely dry and humid habitats with considerable tolerance of temperature fluctuations. It is photophilous, shade intolerant and can tolerate well winds, drought and salty soils.

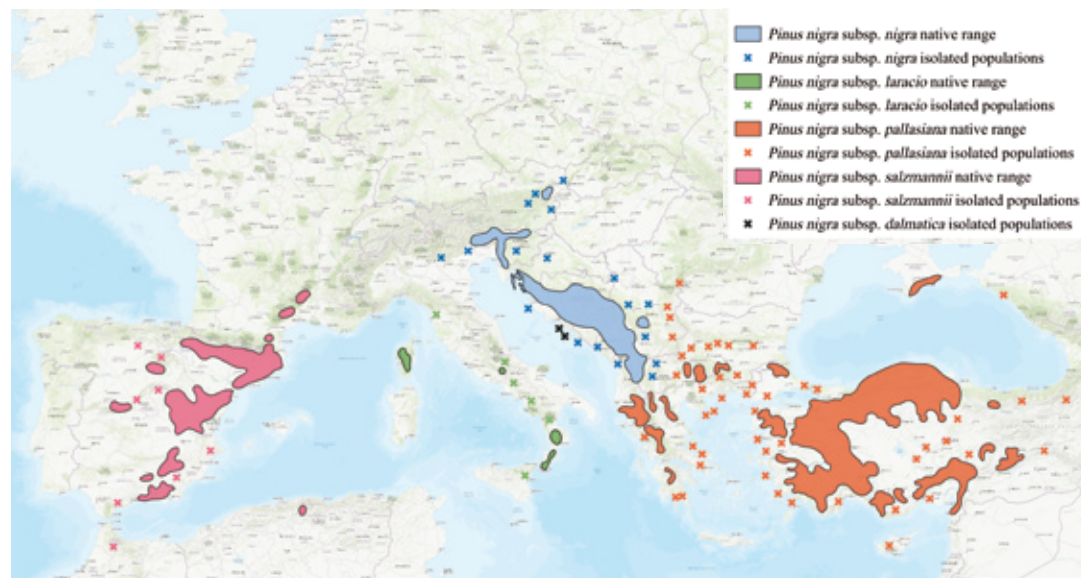


Figure 4.1.1. Black pine distribution range (EUFORGEN 2009, www.euforgen.org).

Pinus pinea L. - Biology and area distribution

Stone pine (*Pinus pinea* L.), also known as umbrella pine, is an evergreen conifer native to the Mediterranean region, distributed from Portugal to Syria and along some coastal areas of the Black Sea (Farjon and Filer, 2013). Its original natural distribution is challenging to determine due to extensive cultivation and diffusion since pre-Roman times, making it difficult to distinguish indigenous areas from those where it was planted. Due to its economic importance, human activity has significantly influenced its current geographical and genetic diversity.

Stone pine is widely cultivated in Spain, Portugal, Italy, and Turkey for purposes such as fruit and wood production, environmental protection, and amenity planting. It has also been introduced successfully to North Africa, Argentina, and South Africa (Bussotti, 1996).

Stone pine is a medium-sized tree, growing 25 to 30 meters with trunks exceeding 2 meters in diameter. The crown is globose and shrubby in youth, becoming umbrella-shaped in mid-age and flat and broad in maturity. The trunk is often short with numerous upward-angled branches with foliage near to the ends. The bark has a complex structure. It is ash grey and fissured in young trees, later it is reddish brown and separated by deep, longitudinal fissures between long grey and flat scaly plates. Buds are about 1 cm long, with brown scales. Needles are bright green, in fascicles of two, on average 8-15 cm long with a sharp apex and stomata on each side. They persist on the tree for 2-3 years. Stone pine is monoecious species. Reproductive maturity in isolated trees begins when trees are 15-20 years of age, and in forest stands when trees are about 20-30 years of age. Yellow pollen catkins

are located in clusters at the base of the seasons shoot and ovulate cones are erect and about 2 cm long. Pollination is anemophilous, occurring from May to June when the pollen is released in great quantity. Fertilization takes place two years post-pollination, with cones maturing in the third year. Mature cones, which are 8-14 cm long, broadly ovoid, sessile, and isolated, remain attached for several years post-opening. Seeds are pale brown, covered with a black powder, 15-20 mm long, heavy, with easily detachable wings that are ineffective for wind dispersal. Stone pine exhibits mast seeding with significant variation in seed production (Eckenwalder, 2009; Johnson and More, 2006).

Stone pine occupies a broad range of climate and soil conditions along the Mediterranean basin. It can be found from sea level up to 500-600 m in the northern Mediterranean and up to 800-1400 m in the east Mediterranean. It predominantly forms pure stands, naturally regenerating by seeds. Stands are found within the thermo- and meso-Mediterranean climate zones and subhumid bioclimates, characterized by hot, dry summers and rainy, mild winters. It is light-demanding and prefers acidic or neutral sandy soils although it tolerates slightly calcareous soils (Montero *et al.*, 2008).

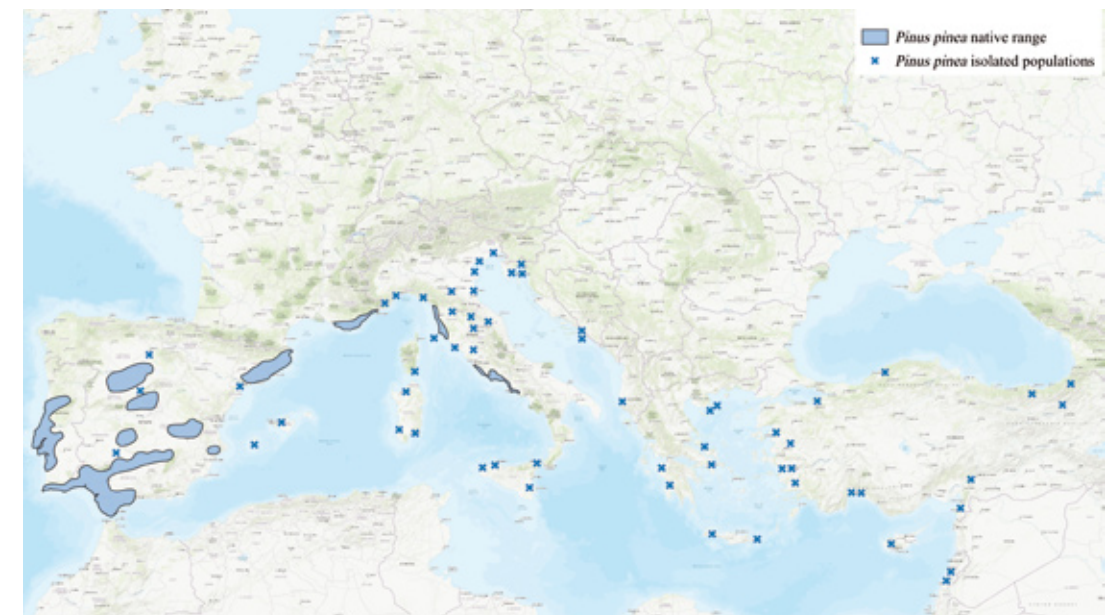


Figure 4.1.2. Stone pine distribution range (EUFORGEN 2009, www.euforgen.org).

Pinus pinaster Aiton - Biology and area distribution

Pinus pinaster Aiton, the maritime pine, is a widespread evergreen tree native to the southern Atlantic Europe region and parts of the western Mediterranean.

It is a medium-sized tree, reaching 20-35 m high. The bark is orange-red, thick and deeply fissured (Iravani and Zolfaghari, 2014). The needles occur mostly in pairs (Idžojtić, 2009), are up to 25 cm long, and bluish-green to distinctly yellowish-green. The cones are ovoid-conic shape, 10-20 cm long, and 5-7 cm broad at the base when closed, green at first, ripening to glossy brown when 24 months old. They open slowly over the next few years, or after being heated by a forest fire, to release the seeds, opening to 8-10 cm broad (Idžojtić, 2013).

The maritime pine range is primarily concentrated in the western Mediterranean Basin and the southern Atlantic coast of Europe. It occurs in the Iberian Peninsula, southern France, western Italy, west-

ern Mediterranean islands, northern Morocco, Algeria and Tunisia. Its presence has expanded due to artificial plantations and naturalization, reaching southwestern France's coast, Adriatic countries, and even northern Europe, including the United Kingdom and Belgium (Jalas and Suominen, 1973; Critchfield and Little, 1966; Pereira, 2002; Farjon and Filer, 2013). Two primary factors have influenced the current fragmented distribution of *P. pinaster*: the discontinuity and altitude of mountain ranges, which isolate even nearby populations, and significant human impact through deforestation and land use changes (Alía and Martín, 2003). Despite these challenges, the species continues to be widely planted and cultivated in various countries, both within and beyond its natural range. It ranges from sea level in coastal lowlands to moderate elevations up to 1600 m in the Iberian Peninsula and island Corsica, and up to about 2000 m in Morocco (Wahid *et al.*, 2006; Farjon, 2010). This elevation range showcases the species' adaptability to different altitudes and climatic conditions. Naturally, it grows in warm temperate areas with oceanic climatic influences, particularly in humid and sub-humid regions with annual rainfall exceeding 600 mm. However, it can survive in regions with only 400 mm of annual precipitation if there is adequate atmospheric moisture. It is not tolerant to shade and exhibits preference for siliceous soils with a coarse texture, especially sandy soils, dunes and other poor substrates (Viñas *et al.*, 2016). Table 4.1.1 reports the list of the sites for *Pinus* spp. of the LIFE SySTEMiC project.

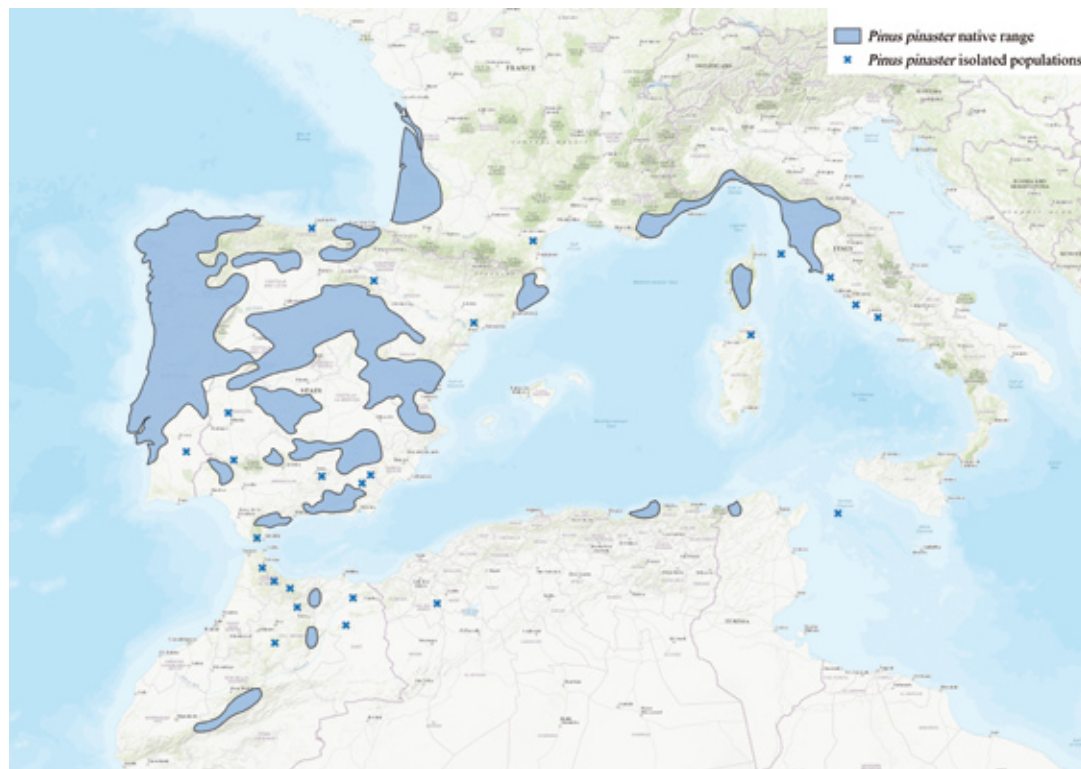


Figure 4.1.3. Maritime pine distribution range (EUFORGEN 2009, www.euforgen.org).

Table 4.1.1. List of the sites for *Pinus* spp. of the LIFE SySTEMiC project.

Id	Site name	Country	Species	EFT*	Structure	Silvicultural system
08	Terminaccio	Italy	<i>P. pinea</i>	10.1	Even-aged	Clear-cutting and planting
9A	Fossacci	Italy	<i>P. pinea</i>	10.1	Even-aged	Clear-cutting and planting
9B	Fossacci	Italy	<i>P. pinea</i>	10.1	Even-aged	Clear-cutting and planting
9C	Fossacci	Italy	<i>P. pinea</i>	10.1	Even-aged	Clear-cutting and planting
15	Zadar	Croatia	<i>P. pinea</i>	10.1	Even-aged	Clear-cutting and planting
17	Klana	Croatia	<i>P. nigra</i>	3.3	Even-aged	Uniform shelterwood
18	Brač	Croatia	<i>P. nigra</i>	10.2	Even-aged	Irregular shelterwood
19	Pelješac	Croatia	<i>P. pinaster</i>	10.1	Even-aged	Irregular shelterwood
22	Mlake	Slovenia	<i>P. nigra</i>	14.1	Even-aged	Uniform shelterwood
31	Mljet	Croatia	<i>P. pinea</i>	10.1	Even-aged	Uniform shelterwood

* EFT = European Forest Type: 3.3 Alpine Scots pine and Black pine forest; 10.1 Mediterranean pine forest; 10.2 Mediterranean and Anatolian Black pine forest; 10.6 Mediterranean and Anatolian fir forest; 14.1 Plantations of site-native species.

4.2 Forest structure deadwood and tree-related microhabitats

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Forest structure, deadwood and tree-related microhabitats were inventoried in nine sites (Figure 4.2.1).

In each site, a permanent plot with a representative structure of forest management was chosen. The plot had a rectangular shape, and each side was 50 m long (area of 2500 m²). Only when the number of trees of the target species (*Pinus* spp. with diameter at breast height > 2.5 cm) was < 30 trees one more plot was established in the site, until the minimum number of 30 trees was reached.

Detailed protocols for field survey and data elaboration are available on LIFE SySTEMiC web page: <https://www.lifsystemic.eu/>.

Pinus pinea L. was the prevailing tree species (basal area > 75%) in the Sites Fossacci 9A, 9B, 9C and Site 15 - Zadar; in the Site 31 - Mljet, *Pinus pinea* L. was mixed with *Pinus halepensis* Mill. and other secondary tree species (*Cupressus sempervirens* L.). *Pinus nigra* J.F. Arnold was the prevailing tree species in Sites 17 - Klana, 18 - Brač and 22 - Mlake. Site 19 - Pelješac was a post-fire pure stand of *Pinus pinaster* Aiton.

Figure 4.2.1. Sites for *Pinus* spp.

The spatial distribution of the trees in the sites is reported in Figure 4.2.2. Figure 4.2.3 shows the stem number-diameter distribution in each site.

Forest structure variables are reported in Table 4.2.1.

In the examined *Pinus pinea* L. stands (Site Fossacci: 9A, 9B, 9C and Sites 15 - Zadar, 31 - Mjliet), all sites were managed with even-aged structures; the basal area ranged between 19 m²/ha and 41 m²/ha, with an average value of 29 m²/ha, and the volume varied between 277 m³/ha and 370 m³/ha, with an average of 322 m³/ha. Forest structure diversity, represented by the coefficient of variation of DBH and the coefficient of variation of heights, was 35.6% and 26.5%, respectively.

Pinus nigra J.F. Arnold stands (Sites 17 - Klana, 18 - Brač, 22 - Mlake) were all managed with an even-aged structure. The basal area ranged between 29 m²/ha and 51 m²/ha, with an average value of 41 m²/ha, and the volume varied between 171 m³/ha and 423 m³/ha, with an average of 322 m³/ha. The coefficient of variation of DBH and the coefficient of variation of heights were 58.5% and 29.0%, respectively.

Pinus pinaster Aiton. Site (19 - Pelješac) had a basal area of 11 m²/ha and a volume of 97 m³/ha.

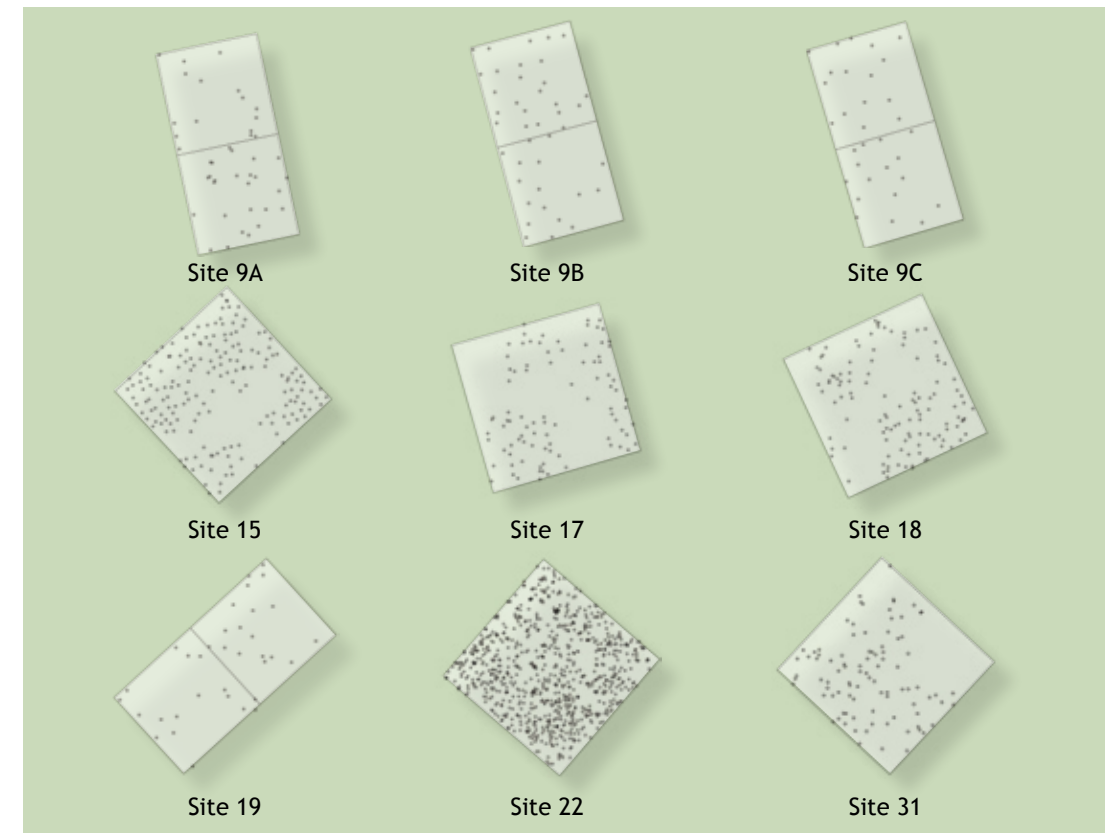


Figure 4.2.2. Spatial distribution of the trees in the sites.

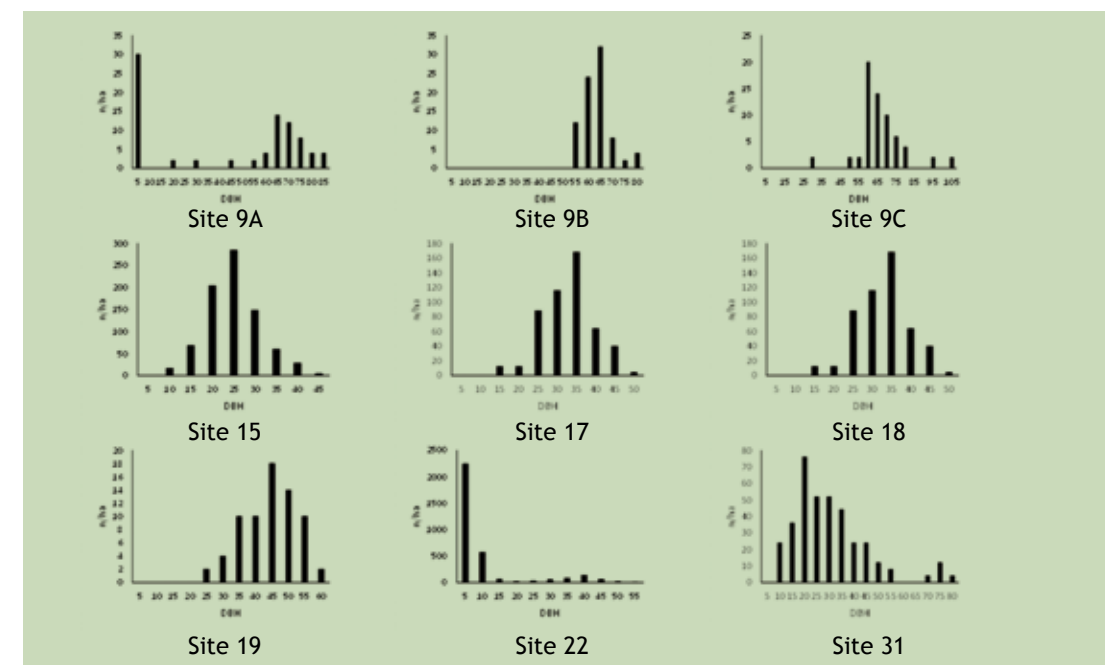


Figure 4.2.3. Stem number-diameter distribution in the sites.

Table 4.2.1. Forest structure variables in the sites. N=number of stems; BA=basal area; V=volume; QMD=quadratic mean diameter (diameter of the mean basal area tree); MH= mean height (height of the mean basal area tree); DH= dominant height (mean height of the 100 trees per hectare with the largest diameters in case of even-aged stand, and mean height of the three tallest trees per hectare in case of uneven-aged stand); SDDBH= standard deviation of DBH; SDH= standard deviation of total tree heights; CVDBH= coefficient of variation of DBH; CVH= coefficient of variation of total tree heights.

Id	N	BA	V	QMD	MH	DH	SDDBH	SDH	CVDBH	CVH
	n/ha	m ² /ha	m ³ /ha	cm	m	m	cm	m	%	%
9A	84	19.2	283.3	53.9	25.7	26.9	32.2	12.8	74.0	70.3
9B	82	26.1	365.7	63.7	26.6	26.4	5.9	3.2	9.3	12.0
9C	64	23.1	315.8	67.9	26.9	26.8	12.9	4.0	19.4	15.3
15	812	41.1	276.7	25.4	9.2	9.5	6.1	0.6	24.9	6.3
17	504	43.7	423.0	33.2	17.6	18.7	6.7	1.8	20.5	10.7
18	532	28.5	170.9	26.1	9.9	10.9	9.5	2.0	39.2	21.0
19	70	11.3	96.5	45.3	16.2	16.0	8.3	3.2	18.6	19.9
22	3256	51.3	372.5	14.2	14.7	23.3	10.7	5.3	115.9	55.4
31	372	33.6	369.7	33.9	14.1	16.4	15.2	3.6	50.3	28.7

For each site, the amount of deadwood is reported in Table 4.2.2. In the *Pinus pinea* L. stands (Sites in Fossacci: 9A, 9B, 9C and Sites 15 - Zadar, 31 - Mjliet), the total volume of deadwood ranged between 6 m³/ha and 20 m³/ha, with an average value of 12 m³/ha, which was mostly represented by snags (49%), other lying deadwood pieces (19%), stumps (17%), and downed dead trees (16%).

Pinus nigra J.F. Arnold stands (Sites 17 - Klana, 18 - Brač, 22 Mlake) had an average volume of deadwood of 21 m³/ha. In the *Pinus pinaster* Aiton. Site (19 - Pelješac) the amount of deadwood was 42 m³/ha.

Table 4.2.2. Volume of deadwood in the sites.

Id	Standing dead trees (including snags)	Downed dead trees	Other lying dead wood pieces	Stumps	Total
	m ³ /ha	m ³ /ha	m ³ /ha	m ³ /ha	m ³ /ha
9A	12.8	0.0	1.9	2.7	17.4
9B	0.0	0.0	1.6	4.8	6.4
9C	0.0	0.0	4.8	1.5	6.3
15	12.3	0.0	0.1	0.0	12.4
17	1.3	0.8	1.1	2.2	5.4
18	4.5	0.1	0.0	0.0	4.6
19	5.4	13.7	21.1	1.5	41.7
22	7.2	32.3	9.5	2.9	51.8
31	5.0	9.7	3.2	1.6	19.5

The frequency of the tree-related microhabitats in each site, represented as the percentage of forms of microhabitats, is reported in Figure 4.2.4. Deadwood was the most common form of microhabitats in *Pinus pinea* L. stands, especially in the sites of Fossacci 9A, 9B and 9C. Deadwood, epiphytes (Site 18 - Brač) and injuries and wounds (Site 22 - Mlake) were common in the *Pinus nigra* J.F. Arnold stands. Cavities, injuries and wounds and other forms of microhabitats were almost equally represented in the *Pinus pinaster* Aiton. Site (19 - Pelješac).

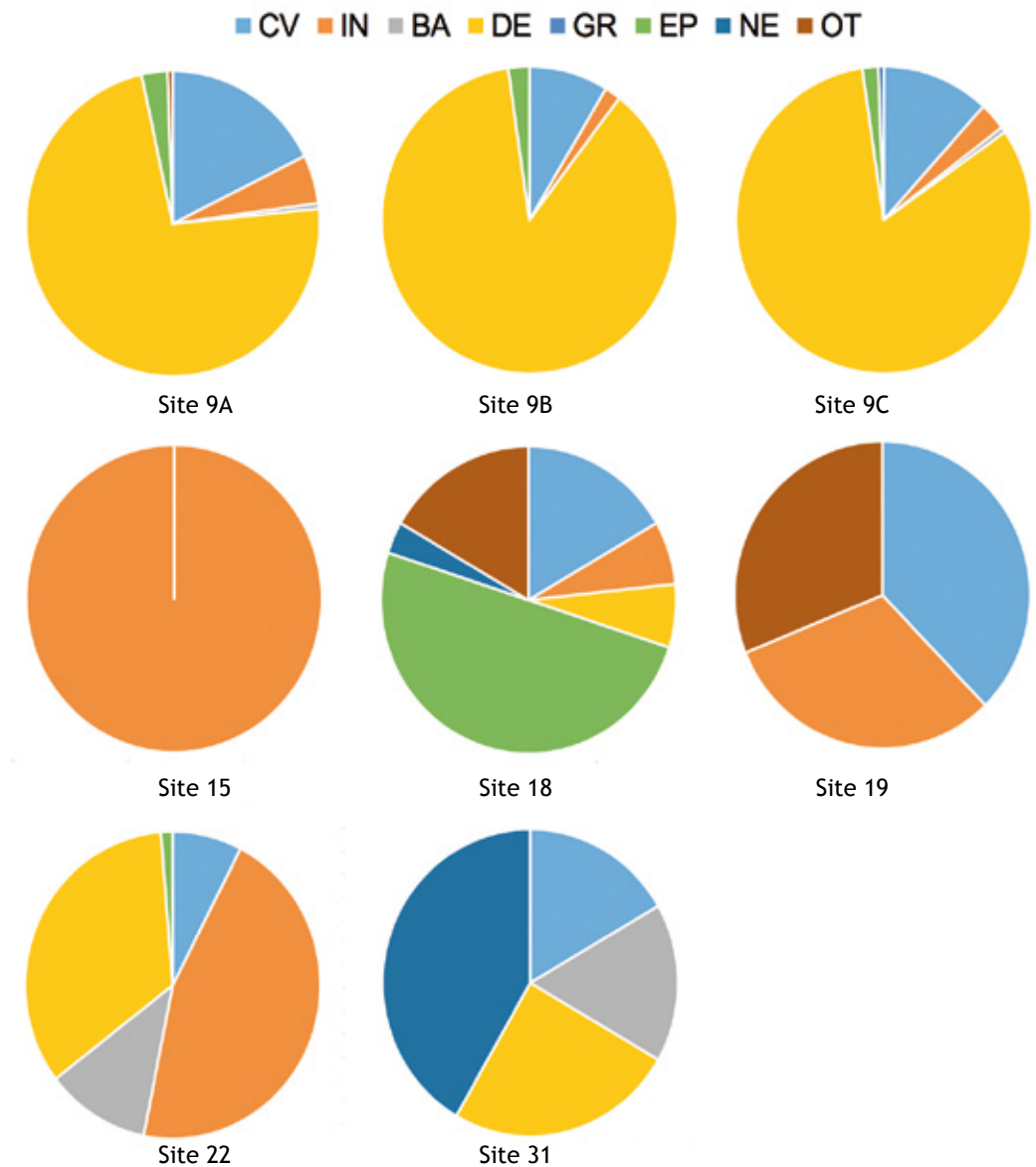


Figure 4.2.4. Tree-related microhabitats in the sites (percentage of forms of microhabitats) (CV = Cavities; IN = Injuries and wounds; BA = Bark; DE = Deadwood; GR = Deformation / growth form; EP = Epiphytes; NE = Nests; OT = Other).



Figure 4.2.5. Deadwood in the Site 9A - Fossacci.



Figure 4.2.6. Natural regeneration of *Pinus pinea* L. in the Site 9B - Fossacci.

4.3 Landscape genomics

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The genus *Pinus* spp. L. includes over 100 species, with many providing significant ecosystem services in the northern hemisphere, particularly timber. Despite its widespread distribution, *Pinus pinea* L. exhibits very low genetic variation (Pinzauti *et al.*, 2012) but demonstrates high phenotypic plasticity (Chambel *et al.*, 2007; Carrasquinho and Gonçalves, 2013). In contrast, *Pinus nigra* J.F. Arnold, or the European black pine, has a fragmented distribution from North Africa to the Northern Mediterranean and east to the Black Sea and Crimea (Eckert and Hall, 2006; Gausson *et al.*, 1964; Scotti-Saintagne *et al.*, 2019). Unlike *P. pinea*, *P. nigra* shows high genetic diversity within populations and low diversity among them (Fady and Conord, 2010; Scotti-Saintagne *et al.*, 2019), a pattern attributed to historically high gene flow over long distances (Kremer *et al.*, 2012; Scotti-Saintagne *et al.*, 2019). The genetic diversity of populations could be important to mitigating the effects of climate change (Mosca *et al.*, 2012) and achieving adaptation through the selection of potentially beneficial alleles present in the populations (Barret *et al.*, 2008). Understanding the dynamics and mechanisms of local adaptation in natural populations provides basis for predicting responses to environmental changes, including those associated with global climate change. Local adaptation is expected to alter the allele frequencies of genes that influence fitness in particular environments (Lefèvre *et al.*, 2014). Therefore, understanding the relationship between genomes and adaptive phenotypic characteristics and the extent to which they are influenced by the environment may be essential for predicting the fate of tree species coping with climate change (Babst *et al.*, 2019; Alberto *et al.*, 2013). In this context, landscape genomics has emerged as a flexible analytical framework for understanding the interactions between environmental heterogeneity and adaptive genetic variation in natural populations (Balkenhol *et al.*, 2016). We used Landscape Genomics approaches to analyse the neutral and adaptive component of genetic diversity to highlight possible patterns of local adaptation in the populations analysed. Therefore, we correlated adaptive molecular markers (SNPs) with bioclimatic indicators. Nuclear microsatellite markers (nSSRs) were analysed as a measure of neutral genetic variation and structure of the studied populations. Single Nucleotide Polymorphism (SNPs) genotyped using a target re-sequencing approach of candidate genome regions were analysed as a measure of adaptive genetic variation of the studied population (Figures 4.3.1 and 4.3.2). As a result of *P. pinea* target re-sequencing about 500 SNPs were called in 28 genome regions relevant for response to one or more abiotic stresses (results reported in deliverable Action B1: SNP road-map of each study site). In addition, we investigated the distribution of these SNPs at the population level, and we have designed road-maps for each target species to observe their spatial distribution among all the analysed populations (Figure 4.3.1).

As stated in bibliography (Pinzauti *et al.*, 2012), *P. pinea* is characterized by low genetic diversity. In Figure 4.3.1., it is possible to observe a low number of site-specific and national-specific SNPs for all the pine sites studied. However, it is interesting to note the presence of a higher number of site-specific SNPs in Site 09 (Fossacci) and in Site 31 (Mljet). All the sites analysed are located in a bioclimatic region characterized by mediterranean climatic conditions.

The observed site-specific SNPs set could be interpreted as a sign of adaptation to a Mediterranean basin bio-climatic regime.

As a result of *P. nigra* target re-sequencing about 2000 SNPs were observed in 21 genome regions relevant for response to one or more abiotic stresses (results reported in deliverable Action B1: SNP road-map of each study site). As reported in Figure 4.3.2, we have designed road-maps for each target species to observe their spatial distribution among all the analysed populations.

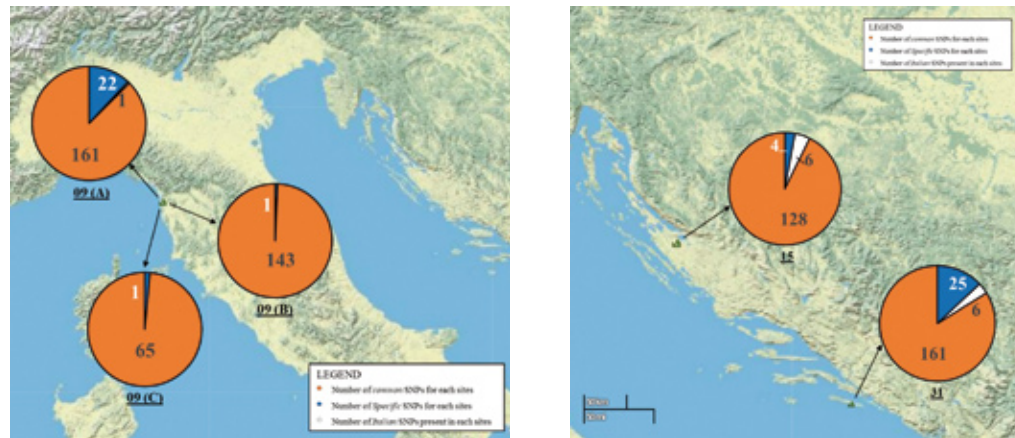


Figure 4.3.1. The road-map of *Pinus pinea* L. SNPs distribution in LIFE SySTEMiC sites of Italy (A) and Croatia-Slovenia (B) is depicted in the figure. The figure shows the number of certain specific subsets of SNPs for each site. The data types are sorted by colour. The colours represent the following SNPs subset: The set of SNPs present in that site which are common to at least two sites of interest (orange); the number of unique SNPs specific to that site (blue) and the number of SNPs present in that site, which are specific to the country of origin (white). Below each graph, the identifying number of the corresponding site is displayed (in bold and underlined).

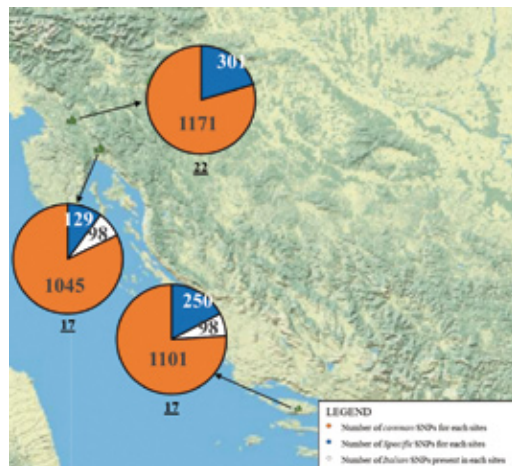


Figure 4.3.2. The road-map of *P. nigra* SNPs distribution in LIFE SySTEMiC sites is depicted in the figure. The figure shows the number of certain specific subsets of SNPs for each site. The data types are sorted by colour. The colours represent the following SNPs subset: The set of SNPs present in that site which are common to at least two sites of interest (orange); the number of unique SNPs specific to that site (white) and the number of SNPs present in that site, which are specific to the country of origin (blue). Below each graph, the identifying number of the corresponding site is displayed (in bold and underlined).

Unlike *P. pinea*, *P. nigra* shows high genetic diversity within populations and low diversity among them (Fady and Conord, 2010; Scotti-Saintagne *et al.*, 2019). In Figure 4.3.2, it is possible to observe a high number of site-specific and national-specific SNPs for all the black pine sites studied. Despite the different bioclimatic region of each analysed site, the high number of site-specific SNPs reported could be interpreted as a sign of adaptation to local environment.

For each site, twelve bio-climatic indicators were selected from the existing literature (Flint *et al.*, 2013; Gugger *et al.*, 2016, 2021; Pluess *et al.*, 2016). To identify local adaptation patterns of the target species, we conducted GEA analyses on two levels: global and site-specific analysis. The global analysis allowed us to identify possible patterns of adaptation to the bioclimatic conditions that characterize the range of Pine spp. The results of the analysis showed the existence of three different clusters for *P. pinea*, and four clusters for *P. nigra*, present in Italy, Croatia and Slovenia. Additionally, an even more interesting finding is the presence of association between some allelic variants and the mean values of the 12 bioclimatic indicators considered for these analyses: 39 SNPs for *P. pinea* and 14 for *P. nigra* (as reported in Deliverable Action B1: Production of maps of spatial distribution of genetic diversity and of correlation between allele distribution and environmental variation). The presence of these associations could be interpreted as adaptation genotype of *Pinus* spp. spread in the Central European range. Of particular interest was finding some site-specific associated allelic variants. The presence of these allelic variants could be correlated with the local rather than regional pattern of adaptation. In the environmental association analysis (EAA) it is important to account for neutral genetic structure (Rellstab *et al.*, 2015), as neutral genetic structure can produce patterns like those expected under non-neutral processes (Excoffier and Ray 2008; Excoffier *et al.*, 2009; Sillanpää, 2011). Furthermore, the genetic structure of populations was analysed using STRUCTURE (Pritchard *et al.*, 2000) and GENELAND software (Guillot 2008). For *P. pinea*, we found a low to moderate number of correlated allelic variants in each site. We found that Site 09 - Fossacci presented a higher number of SNPs correlated with bioclimatic indicators (20 SNPs). The presence of these allelic variants associated with bioclimatic indicators that best characterize the local environment is important for adaptation. Despite the highest number of correlated SNPs reported for Site 09, the spatial genetic structure is simplified (1 cluster, results reported in deliverable Action B1: Production of maps of spatial distribution of genetic diversity and of correlation between allele distribution and environmental variation). Instead, as reported in Figure 4.3.3, we have observed a less simplified spatial genetic structure and a high number of correlated SNPs in Site 31 - Mljet.

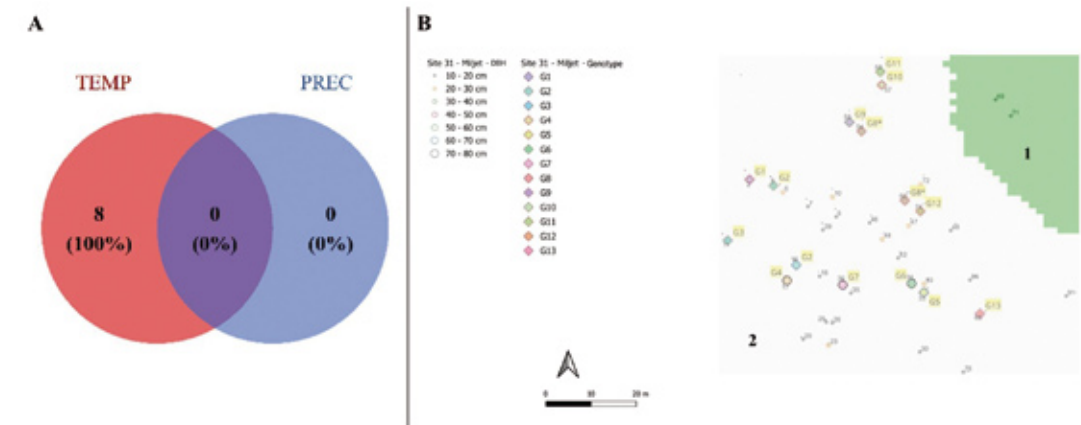


Figure 4.3.3. LFMM analysis results and genotype distribution map of Site 31 - Mljet. (A) Venn diagram showed the overlapping between SNP associated with temperature-related and precipitation-related bioclimatic indicators, as a result of LFMM analysis. (B) Spatial distribution of genotype and spatial organization in 3 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter is characterized by coloured circles according to the genotype observed. Identical colours mean identical genotypes.

Based on the results obtained, it is possible to assume that favouring the natural regeneration of *P. pinea* could produce good results in terms of genetic diversity and adaptation of populations to future climatic changes. The presence of a high number of SNPs associated with adaptation to bioclimatic indicators at these sites could be correlated with the neutral genetic structure observed for these sites (Aravanopoulos, 2018; Paffetti *et al.*, 2012; Stiers *et al.*, 2018).

For *P. nigra*, a completely different situation was observed. Comparing managed to unmanaged sites, we have observed a general complex spatial genetic structure with reduced number of correlated SNPs. Site 18 - Brač (Figure 4.3.4) and Site 22 - Mlake, reported the highest values of adaptive genetic diversity.

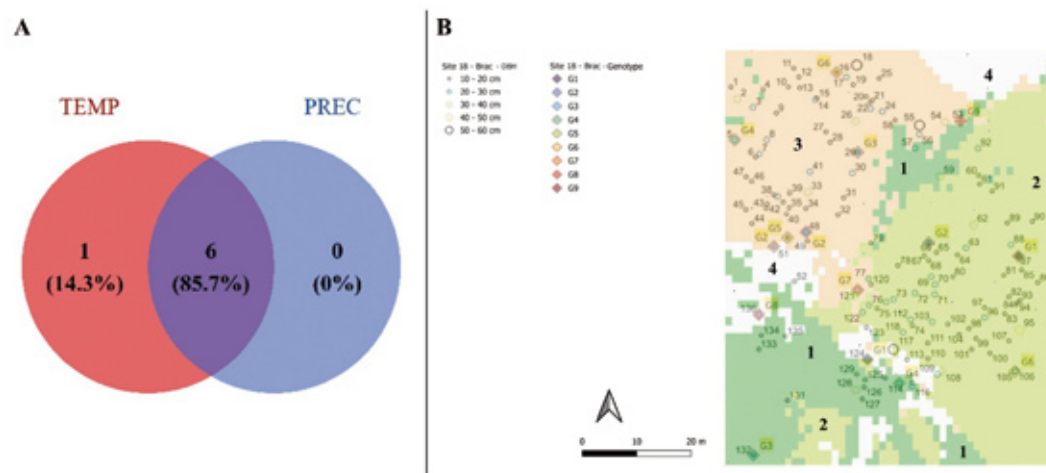


Figure 4.3.4. LFMM analysis results and genotype distribution map of Site 18 - Brač. (A) Venn diagram showed the overlapping between SNPs associated with temperature-related and precipitation-related bioclimatic indicators, as a result of LFMM analysis. (B) Spatial distribution of genotype and spatial organization in 4 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter is characterized by coloured circles according to the genotype observed. Identical colours mean identical genotypes.

The presence of these allelic variants associated with bioclimatic indicators that best characterize the local environment is important. We found that managed sites presented the highest number of SNPs correlated with bioclimatic indicators.

Based on the results obtained, it is possible to assume that favouring of natural regeneration of *P. nigra* could be important to improve genetic diversity and adaptation of populations to future climatic conditions. Less impactful management types, such as individual tree selection, appear to report population with a high number of allelic variants associated with response to bioclimatic indicators. Instead, simplified spatial genetic structure and low number of correlated SNPs were reported for unmanaged stands. The results reported in this study could be important in silvicultural management planning, where knowledge of genetic variability from an adaptive perspective could help decision-making processes. This is important to preserve the current Forest Genetics Resources (FGR), but also to enrich the existing stand with potentially favourable genotypes.

4.4 GenBioSilvi model

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The significance of all species in forest ecosystems cannot be overstated; from fungi and small mammals to invertebrates, each plays a vital role in maintaining forest productivity and ecosystem functioning. Conserving forest biodiversity is essential for upholding ecological processes like decomposition, nutrient and hydrological cycles, succession, and natural regeneration, thereby promoting ecosystem stability. To ensure biodiversity quality, it is crucial to protect or enhance the intricate patterns and processes fostering heterogeneity within forest ecosystems (Spanos *et al.*, 2007). Biodiversity loss is occurring on local, regional, national, and global scales (Fussi *et al.*, 2016). This decline is primarily driven by human activities and climate change, especially through factors such as habitat destruction, reduction in species populations, overexploitation of resources, introduction of invasive species, and an increase in extreme weather events (Hoban *et al.*, 2020).

Biodiversity conservation is one of the main goals of ecologically sustainable forestry. In this regard, it is necessary to develop a predictive model for sustainable forest management (SFM). The objective of the project LIFE SYSTEMiC is to develop genetic and forest indicators in different European Forest Types (EFTs) to implement the predictive model for sustainable forest management, namely GenBioSilvi predictive model.

This model is designed to integrate multiple indicators, including genetic diversity, forest structure, deadwood, soil diversity, and microhabitat conditions (see the deliverable Action B2 GenBioSilvi model). These indicators are calculated for each species involved in the project. In this chapter, we will specifically focus on *Pinus* spp. By combining these diverse indicators, the GenBioSilvi model aims to provide a comprehensive tool for sustainable forest management, supporting forest ecosystems in responding to climate change.

Several studies have demonstrated that *Pinus pinea* L. exhibits significantly lower genetic variability compared to other conifer species (Vendramin *et al.*, 2008, Carrasquinho *et al.*, 2013, Sáez-Laguna *et al.*, 2014, Mutke *et al.*, 2019). This reduced genetic variability is partly attributed to historical population bottlenecks and limited gene flow, as well as the species' clonal propagation and patchy distribution across the Mediterranean region. Despite this low genetic diversity, *P. pinea* exhibits high phenotypic plasticity, allowing it to adapt to various environmental conditions through mechanisms such as epigenetic modifications. Based on the results obtained, we can assume that there is low genetic diversity in all sites but we can't conclude that there is significant genetic diversity between the sites even though they differ in management types. In this project, we studied three types of managed forests (Clearcutting and planting, irregular shelterwood system, and individual tree selection system). Analyzing the pattern of genetic diversity distribution based on nSSR data, we observed that all the sites were characterized by simplified spatial genetic structure (1 or 2 clusters). This can be attributed to the low genetic diversity that characterizes *P. pinea*, as reported in the bibliography.

Despite the low genetic diversity reported by observing SSRs-based distribution results reported in Deliverable Action B2 of the GenBioSilvi model, we observed high genetic diversity by observing SNPs in candidate genes for abiotic stress response. In particular, Site 09 - Fossacci, reported the highest values of adaptive genetic diversity.

The presence of these allelic variants associated with bioclimatic indicators that best characterize local environment is important. We found that Site 09 - Fossacci presented a higher number of SNPs correlated with bioclimatic indicators.

Based on the results obtained, it is possible to assume that stimulation of natural regeneration of *P. pinea* could produce good results in terms of genetic diversity and adaptation of populations to future environmental changes.

Stand structure is a key element in assessing ecological functions and services in forest ecosystems. To describe biodiversity, we observed dendrometric data to define the forest structure of the analysed sites. Thanks to this data, we can assume that the best structure observed is that characteristic of a multi-layered forest with the presence of regeneration. We found these characteristics in all managed sites except Site 15 - Zadar. The Stone pine population analysed presents a small to consistent layer of regeneration and a more widespread layer of adult trees. A multi-layered uneven-aged population supports the presence of natural regeneration because it shows gaps in canopy cover that create ideal conditions for the establishment of natural regeneration. Furthermore, a multi-layered forest increases the probability of sporadic species occurrence. In addition, multi-layered forest allows high pollen dispersal, promoting genetic diversity. Deadwood plays crucial functional roles such as in nutrient cycling and as a carbon sink. The presence of deadwood can promote germination and seedling development of tree species, acting as nursery sites and thus contributing to forest renewal. Regarding the amount of deadwood, the best situation is observed in unmanaged sites. Site 09 - Fossacci showed a higher volume of deadwood. In the past, deadwood debris in managed sites were promptly removed for security purposes (pathogen proliferation, fire danger) and were synonymous with mismanagement. Deadwood provides an important microhabitat for the development and conservation of species important to the forest ecosystem. In addition to deadwood, we observed other indicators related to saproxylic microhabitats. The frequency of epixylic microhabitats is lower than saproxylic in all observed populations. Epixylic are used as indicators to assess ecosystem conditions. The presence of these microhabitats is an important source of biodiversity. Based on the obtained results, management types that allow for complex forest structures characteristic of uneven-aged and multilayered stands increase the probability of observing different microhabitat forms.

Pinus nigra J.F. Arnold has been commonly used for reforesting areas with challenging soils and severe climatic conditions (Dias *et al.*, 2020). Based on the results reported in Deliverable Action B2 of the GenBioSilvi model, we can assume that there is high genetic diversity in all sites but we can't conclude that there is significant genetic diversity between the sites even though they differ in management types. In this project, we studied two managed forests (individual tree selection system) and one unmanaged forest. Analyzing the pattern of genetic diversity distribution based on nSSR data, we observed that the unmanaged site was characterized by a simplified spatial genetic structure (2 clusters). This can be attributed to the type of management that influences the regeneration potential. Moreover, we observed high genetic diversity by observing SNPs in candidate genes for abiotic stress response. In particular, Site 22 - Mlake, reported the highest values of adaptive genetic diversity. The presence of these allelic variants associated with bioclimatic indicators that best characterize the local environment is important. We found that managed sites presented the highest number of SNPs correlated with bioclimatic indicators. Based on the results obtained, it is possible to assume that stimulation of natural regeneration of *Pinus nigra* J.F. Arnold could produce good results in terms of genetic diversity and adaptation of populations to future environmental changes. The analysed sites exhibit a bell-shaped diameter class distribution and a vertical mono-bi stratified structure, lacking regeneration of target species. Notably, there is an abundance of regeneration of non-target, hard deciduous species across all sites. These characteristics were consistently observed in every location studied. Regarding the amount of deadwood, the best situation is observed in unmanaged sites. Site 22 - Mlake which showed the highest volume of deadwood. In addition to deadwood, we observed other indicators related to saproxylic microhabitats. We observed two distinct scenarios in managed sites: at Site 22 - Mlake, the frequency of epixylic microhabitats was lower than that of saproxylic microhabitats, whereas at Site 18 - Brač, the frequency of saproxylic microhabitats was lower than that of epixylic microhabitats. Epixylic are used as indicators to assess ecosystem conditions. The presence of these microhabitats is an important source of biodiversity. Based on the obtained results,

the site managed according to tree selective thinning increases the probability of observing different microhabitat forms. Generally, we can assume all the analysed sites are pure black pine stands. However, Site 22 - Mlake presented the highest number of non-target species with strong renovation. Black pine is a species that is consociated with other species that show optimal growth under the same conditions (*Quercus ilex* L., *Quercus cerris* L., and *Pinus halapensis* Mill.).

The development of the form for *Pinus nigra* J.F. Arnold and *Pinus pinea* L. was not pursued due to an insufficient number of sites. This limitation didn't allow us to adequately represent and differentiate multiple scenarios, essential for capturing the biodiversity of these species comprehensively and accurately.

Similarly, *Pinus pinaster* Aiton within the genus *Pinus* underwent analysis, mirroring the methodologies employed for *P. nigra* and *P. pinea*. However, the limited number of available sites (1 each) posed a challenge in obtaining statistically significant results.

4.5 Recommendations for Sustainable Forest Management

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Pinus pinea L.

Stone pine (*Pinus pinea* L.) is a Mediterranean species that spread from the Portuguese Atlantic coast to the shores of the Black Sea and the slopes of Mount Lebanon (Quézel and Médail, 2003). The most important distribution areas are in Spain (470,000 ha), Portugal, (80,000 ha), Turkey (50,000 ha), and Italy (46,000 ha). More than half of this area is the result of afforestation (Martinez *et al.*, 2004).

In Italy, stone pine forests have characterized the coastal landscape for approximately five centuries. In Tuscany (Central Italy), a large part of stone pine forests was planted to stabilize coastal sandy soils after reclamation works, begun by the Medici grand duchy in the 16th century and continued in the 18th and 19th century under the Lorena family. Since then, pine forests have produced important goods and services that have contributed to the socio-economic development of the coastal area and also to the wellbeing of the inhabitants. The pine forests have stabilized dunes and protected agricultural lands from sea winds; provided wood products which, in some areas, continues as the transformation of wood into woodchips; produced pine nuts, a highly valued product by the food industry which has faced a crisis in Italy with the appearance of the western conifer seed bug (*Leptoglossus occidentalis* Heidemann) in 1999; supported production of other non-wood products such as turpentine and truffles; and offered pasture and shelter for sheep and cattle (Del Perugia *et al.*, 2017).

Coastal pine forests provide important habitats for the conservation of plant and animal species of naturalistic interest and for this reason they are often included into sites of the Natura 2000 network.

These woodland associations have, over the centuries, carried out and continue to carry out historical-cultural, social, and landscape functions of increasing significance because they have contributed to attracting intensive touristic-recreational activities.

The functionality and persistence of Stone pine forests is, however, threatened by both biotic and environmental adversities. Coastal erosion is a threat for pine forests facing the coast. Where the phenomenon is intense, the first area of erosion is the protective stretch of maritime pines with subsequent

exposure of the stone pine canopy to sea winds which cause it to dry out, followed by direct deterioration of the trees themselves. Erosion can also lead to infiltration of seawater into the soil and salinization of the phreatic layer, causing stress to the pine forest and undergrowth, especially in interdunal areas. Reclamation canals, if they are not maintained efficiently, cannot properly regulate the outflow of water and in rainy periods standing water can accumulate, thus causing further stress to the forest floor. These facts make the pine forest-Mediterranean maquis system particularly vulnerable to attack by parasites. The principal phytopathologies include *Tomicus destruens* Woll, which is responsible for pine forest deterioration, the previously cited western conifer seed bug, responsible for the collapse of pine nut production, the non-native pine tortoise scale *Toumeyella parvicornis* (Hemiptera: Coccidae) (Garonna *et al.*, 2018), and *Heterobasidion irregulare* Garbel. & Orosina, an agent of root rot in pines (Gonthier *et al.*, 2015).

Stone pine stands are among the ecosystems most vulnerable to climate change, and the current increase in drought frequency in the Mediterranean Basin has been shown to negatively impact their long-term establishment (Mazza and Manetti, 2013; Piraino 2020; Mechergui *et al.*, 2021). Furthermore, the risk of fire along the coast is high, especially in summer.

Stone pine forest stands are mostly of artificial origin and usually present even-aged structures with a rotation length ranging between 80 and 120 years depending on site index. Traditional even-aged stand management in *Pinus pinea* L. is based on clearcuttings, which was maintained up to the end of the decade of 1970's (Calama *et al.*, 2017) and then replaced with the uniform shelterwood system to get a close-to-nature regeneration system (Pardos *et al.*, 2015; Calama *et al.*, 2017; Mechergui *et al.*, 2021). However, selection cutting with diametrical criteria is fairly widespread in multiage complex structures (Barbeito *et al.*, 2008; Pique-Nicolau *et al.*, 2011).

In Italy, the forest management system for stone pine forests is still based on clearcutting with artificial regeneration used to optimize the production of seeds and/or wood. Today, this system is considered to reduce the environmental and landscape quality, and is often cause of conflicts, especially in protected areas and landscapes. This is one of the reasons behind a lack of active management of coastal Stone pine forests in recent decades, which brought forest owners and managers to ask for new silvicultural models (Del Perugia *et al.*, 2017; Portoghesi *et al.*, 2022).

In the Site 09 - Fossacci of the LIFE SySTEMiC project, we found an even-aged 103 years old *Pinus pinea* L. stand with natural regeneration of pine (Figure 4.5.1). The stem density of the natural regeneration of pine varied between 362 trees/ha in plot C and 688 trees/ha in plot A. Most of the regeneration was



Figure 4.5.1. Site 09 - Fossacci, natural regeneration of *Pinus pinea* L.

represented by seedlings (> 80 % in all plots) and the remaining by saplings (Travaglini *et al.*, 2022). Our results show that natural regeneration of *P. pinea* in the Park of San Rossore is a reachable target, however an adequate management is needed.

Calama *et al.*, (2013) found that the most favourable photosynthetic conditions for Stone pine seed dispersal, germination and seedling emergence are achieved in the midshaded positions, just below the area of the crown, at least in the initial stages of the regeneration process, although parent trees should be progressively removed to release the young pine trees as their light requirements increase. Aggregation of regeneration within or near to the area of crown influence is also explained by the limited seed dispersal capacity of Stone pine (Barbeito *et al.*, 2008).

The studies of Manso *et al.* (2014) and Calama *et al.* (2017) report that the even-aged management practices used for the uniform shelterwood system may be behind the failure to support natural regeneration in Stone pine stands, particularly due to the low stand densities and large gap size occurring during the regeneration period. Indeed, due to incapacity to disperse seeds in large gaps created by intensive seed cut and secondary cuts, large areas remain without regeneration for years after the felling.

Thus, to ensure seed arrival into gaps, thinning schedules should target to densities of about 125-150 stems ha⁻¹ at the beginning of regeneration fellings, and intense fellings (e.g., intense uniform shelterwood system) should be replaced by more gradual fellings (Calama *et al.*, 2017). Simultaneously, it may be necessary to control the density of the understory vegetation (Ciancio *et al.*, 1986). However, as an alternative in those locations with abundant advanced regeneration, a shift towards uneven, multi-aged management by means of group selection system should be applied (Barbeito *et al.*, 2008; Ciancio *et al.*, 2009), also for stimulating gene flow (Mechergui *et al.*, 2021).

Pinus nigra J.F. Arnold

Black pine (*Pinus nigra* J.F. Arnold) can be subdivided into five subspecies: *P. nigra* J.F. Arnold subsp. *nigra*; *P. nigra* subsp. *Salzmannii* (Dunal) Franco; *P. nigra* subsp. *larico* (Poir.) Palib. Ex Maire; *P. nigra* subsp. *dalmatica* (Vis.) Franco; *P. nigra* subsp. *pallasiana* (Lamb.) Holmboe.

Black pine is a pioneer and heliophilous species and, due to its characteristics as a pioneer species, it has been used in reforestation projects for soil protection.

Silviculture of black pines in the Mediterranean Countries varies between clearcutting and various types of clear felling (strip or patch), irregular or uniform shelterwood systems, and selection cuttings.

Clearcutting with planting is usually carried out on 1-3 hectares. Clear felling (strip or patch) with natural regeneration by lateral dissemination is carried out on small areas; natural regeneration by lateral dissemination is facilitated by the burning of utilisation residues and the scarification produced on the soil by the dragging of logs. In the absence of regeneration, planting is used.

When irregular or uniform shelterwood systems are used, the natural regeneration takes place under the shelter and protection of the mature stand.

The small group selection method has been reported for *P. nigra* subsp. *larico* (Poir.) Palib. Ex Maire in Calabria, Southern Italy. This method has contributed to the maintenance of pure pine stands with complex uneven-aged structures in private forests (Ciancio *et al.*, 2006).

Based on the experiences of the LIFE SySTEMiC project, forest management systems based on the natural regeneration of pines are best suited to promote genetic diversity and forest adaptation to future environmental changes.

In case of reforestations carried out for soil protection, renaturalisation is used as a silvicultural and management approach which tends to favour natural evolutionary processes through the system's ability to autonomously increase its complexity and biodiversity (Nocentini, 2006).

Pinus pinaster Aiton

The maritime pine (*Pinus pinaster* Aiton) is a pioneer and a light-demanding species that prefers siliceous soils. Compared to the stone pine (*Pinus pinea* L.), the maritime pine tolerates drought less well, as it has a shallower root system. It is a species that produces fruits very early and regenerates itself easily after fires.

Maritime pine forms pure stands in stations with poor soils, where the pine forest has the structure of a multi-layered forest. In other cases, the regeneration of the pine forest is mainly linked to the repetition of fires. If pine regeneration occurs in abundance after a fire, very dense pine forests with even-aged structure result.

The maritime pine has suffered damage from the *Matsucoccus feytaudi* Ducasse, linked to drought stress. In addition to *Matsucoccus feytaudi* Ducasse, other pathogens include fungi such as *Melaspore pinitorqua* and *Coronarum flaccidum*, wood (*Diorictria silvestrella*) and leaf (*Thaumetopoea pityocampa*) lepidoptera.

Maritime pine forests managed with clearcutting can be renewed naturally or artificially. Natural regeneration can be fostered by burning the utilisation residues containing the cones, which simulates the action of a fire, and very dense young stands can be obtained. Artificial regeneration can take place with the use of seedlings or genetically improved seed. Very dense juveniles require very early tending measures, e.g. 2-4 thinning between the age of 4 and 15 years. Then, thinnings are gradual to avoid isolating the trees with the risk of crashes. Clear felling (strip or patch) with natural regeneration by lateral dissemination is carried out on small areas.

The irregular shelterwood system is used to naturally regenerate the pine stand under the shelter and protection of the mature stand. The individual tree selection system can be used with intervals of 15-20 years to obtain uneven-aged and more complex structures.



5. SUSTAINABLE FOREST MANAGEMENT OF *QUERCUS* spp. (*Quercus robur* L., *Quercus pubescens* Willd., *Quercus ilex* L.)

5.1 Introduction

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Quercus spp.

Oaks are a genus of trees and shrubs in the Fagaceae family. Ecologically, oaks are key species found in areas ranging from the continental parts of the Northern Hemisphere, through Mediterranean semi-deserts, to subtropical rainforests. There are over 600 different species of oaks. Oaks are monoecious and can be either deciduous or evergreen. The oak symbolizes sturdiness, strength, and resilience and is often referred to as the “king of the forest”. They are frequently characterized by their large size and slow growth. Oaks produce a nut called an acorn, which is encased in a cup-shaped cap, matures in the same year, and contains tannic acids that protect it from certain fungi and insects.

Quercus robur L. - Biology and area distribution

The pedunculate oak (*Quercus robur* L.) is one of the most widespread and valuable tree species in Europe, having historically played a significant economic, social, and ecological role (Morić *et al.*, 2018). According to Klepac (1996), the ecological impact of pedunculate oak forests is estimated to be several times greater than their economic impact, with particular emphasis on their anti-erosion and hydrological regulatory functions.

Individual pedunculate oak trees can live for over 1,000 years. These trees typically grow to a height of 30-40 (up to 50) meters and can have a diameter of up to 2.5 meters. They are characterized by a broad, often irregular but well-branched crown. The bark is smooth in youth but later becomes longitudinally fissured with deeper and shallower transverse cracks. The root system features a well-developed taproot that penetrates several meters deep into the soil, with lateral roots spreading outwards.

The buds are covered with spirally arranged light brown scales, with the terminal bud surrounded by several smaller lateral buds. The leaf blades are borne on petioles 2-10 mm long, with leaves varying in size from 8-15 (up to 20) cm in length and 3-10 cm in width. The leaf base can be asymmetrical, rounded, or auriculate. Male flowers are found in catkins 2-5 cm long, while female flowers appear singly or in groups of up to five. The acorn, the fruit of the oak, has longitudinal stripes, is light brown or yellowish, and measures 1.5-5 cm in length and 0.7-2.7 cm in width (Franjić and Škvorc, 2010).

The pedunculate oak is a monoecious, deciduous species. It is also wind-pollinated (anemophilous), sun-loving (heliophilous), and moderate in its moisture requirements (mesophilous). It flowers in April and May, and its acorns mature and fall to the ground in September and October, which is the primary method of reproduction. Various birds and mammals help disperse the seeds over greater distances (Pasta *et al.*, 2016).

The pedunculate oak grows throughout nearly all of Europe, from Norway and Sweden in the north to the Iberian, Apennine, and Balkan Peninsulas, and Turkey in the south (Pasta *et al.*, 2016), even reaching northern Scotland. It also extends to the Caucasus and Asia Minor, covering areas north and east of the range of the sessile oak. The soils on which the pedunculate oak thrives are predominantly fertile clayey or sandy soils, typically moist with high groundwater levels. The pedunculate oak often grows in communities with hornbeam and field ash (Franjić and Škvorc, 2010). The pedunculate oak tolerates winter well, although late spring frosts can cause significant damage to young leaves, ulti-



mately impacting growth. Additionally, because nutrients from the root system are used for the development of new leaves, a year with frost is expected to result in a significantly lower acorn yield.

Additionally, in its habitat, the pedunculate oak prevents waterlogging of the terrain and positively influences the soil water system by maintaining the balance of water status through the process of transpiration.

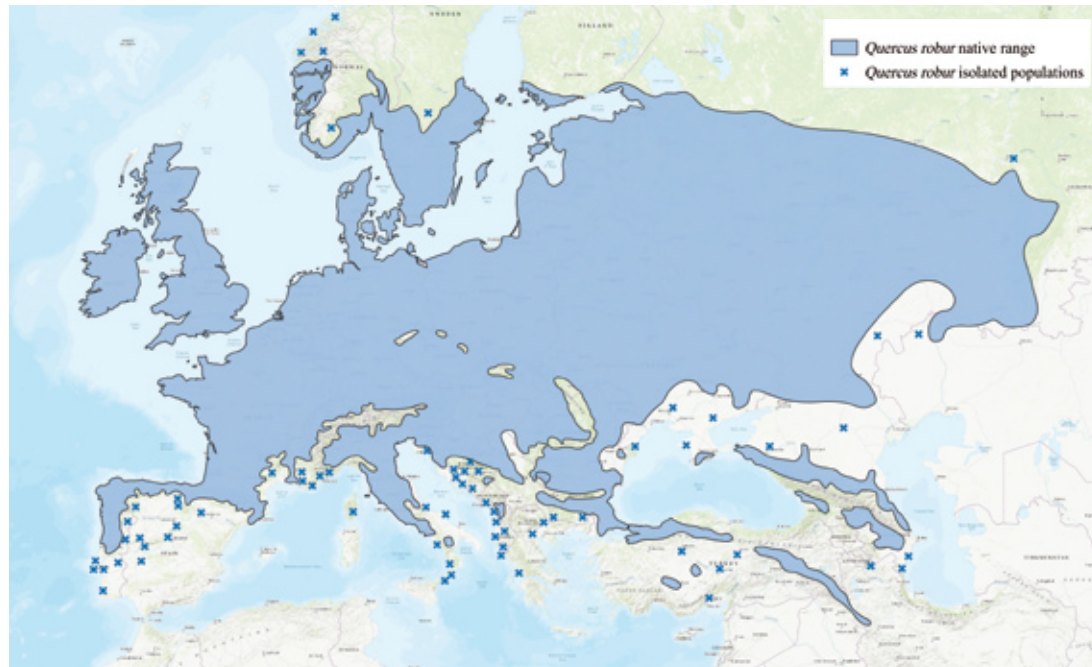


Figure 5.1.1. Pedunculate oak distribution range (EUFORGEN 2009, www.euforgen.org).

Quercus pubescens Willd. - Biology and area distribution

Pubescent Oak (*Quercus pubescens* Willd.) is one of the most widespread tree species in Europe south of the Danube, ranging from the Atlantic coast of France to the Black Sea and Crimea, and extending to the Caucasus and Asia Minor (Tutin *et al.*, 1993). It is a highly polymorphic species with numerous taxa that primarily differ in some morphological characteristics (Franjić and Škvorc, 2010). It predominantly inhabits warmer areas with Mediterranean and sub-Mediterranean climates. Pubescent Oak shows a wide range of altitudes and is the dominant tree species in thermophilic and sub-Mediterranean forests of southeastern Europe (Horváth *et al.*, 1974). It grows on dry and skeletal soils, and in the interior regions on warmer exposures. It can be found individually or in scattered groups, thickets, and other thermophilic groups (Franjić and Škvorc, 2010).

Pubescent Oak is a medium-sized tree reaching up to 20 meters in height and over 100 cm in diameter (Franjić and Škvorc, 2010). The bark is thick, grayish, and deeply fissured. The buds are small and gray-brown. The leaves are alternate, ovate-oblong, and up to 10 cm long. The flowers are unisexual; male flowers are in catkins, while female flowers are solitary or in dense clusters. The fruits are in clusters of 3-4 small acorns on short, hairy stalks and are enclosed by a cupule covering 1/2 to 1/3 of their length (Pasta *et al.*, 2016). It flowers from March to May, but flowering time depends on the altitude. The acorns mature in the autumn of the same year and can germinate immediately under favourable conditions, being significant for animal nutrition (Pasta *et al.*, 2016).

Pubescent Oak is a deciduous, monoecious, anemophilous, xerothermic, and calciphilous species (Franjić and Škvorc, 2010).



Figure 5.1.2. Pubescent oak distribution range (EUFORGEN 2009, www.euforgen.org).

Quercus ilex L. - Biology and area distribution

Quercus ilex L., the holm oak or evergreen oak, is a broad-leaved evergreen tree or shrub native to the Mediterranean basin, where it represents the dominating species in woodlands and maquis vegetation.

The holm oak can grow up to 20-27 m (Schirone *et al.*, 2019), and it is renowned for its resilience and longevity, often living for centuries (Gea-Izquierdo *et al.*, 2011; Praciak *et al.*, 2013). The bark is brownish-black and is lightly fissured into small, square, thin plates (Mitchell, 1974). The leaves are dark green with a woolly lower side, highly variable in shape, typically being narrowly oval or ovate-lanceolate, and measure 3-8 cm in length and 2-4 cm in width (Idžojtić, 2009). The margins are wavy or sinuate, but they can be dentate or in some case spinose on young trees. Flowering is in April and May, during the leafing season. The fruit is an acorn ripening in October and November within the first season, brown in color, 2.5 cm long (Idžojtić, 2013).

The natural distribution of holm oak occurs in the Mediterranean Basin. Across its distribution, two subspecies are identified primarily by variations in leaf morphology: *Quercus ilex* subsp. *rotundifolia* (sometimes referred as *Quercus ilex* subsp. *ballota* or as separate species *Quercus rotundifolia*) has more lanceolate leaves with 6-8 veins and is found in Portugal, southern and southeastern Spain, and Morocco; while *Quercus ilex* subsp. *ilex* has more ovate leaves with 8-9 veins and occurs in the remaining areas (Schwarz, 1993; Praciak *et al.*, 2013). In western regions (the Iberian Peninsula, the Atlantic and Mediterranean coasts of France, the Italian peninsula, the main Mediterranean islands), holm oak forming large pure stands, while in eastern regions (Balkan coasts, Greece, Crete, Black Sea and northern Lebanon) it is more commonly found in mixed stands (Schirone *et al.*, 2019). The altitudinal range is variable, growing from 100-140 m above sea level in the Black Sea area, up to 400-600 m in the Mediterranean, while in Morocco, it grows up to altitudes of 2000-2600 m (Schirone *et al.*, 2019).

The holm oak is a tree able to grow on various soil types and in diverse Mediterranean climates, rang-

ing from semi-arid to very humid conditions with respect to precipitation, and from warm to very cold temperatures at high altitudes, provided the precipitation remains low (Barbero *et al.*, 1992). However, despite its ability to thrive in diverse environments, pure stands of holm oak forests are becoming increasingly rare due to human activities such as deforestation, urbanization, and agricultural expansion over centuries. Table 5.1.1. reports the list of the sites for *Quercus* spp. of the LIFE SySTEMiC project.

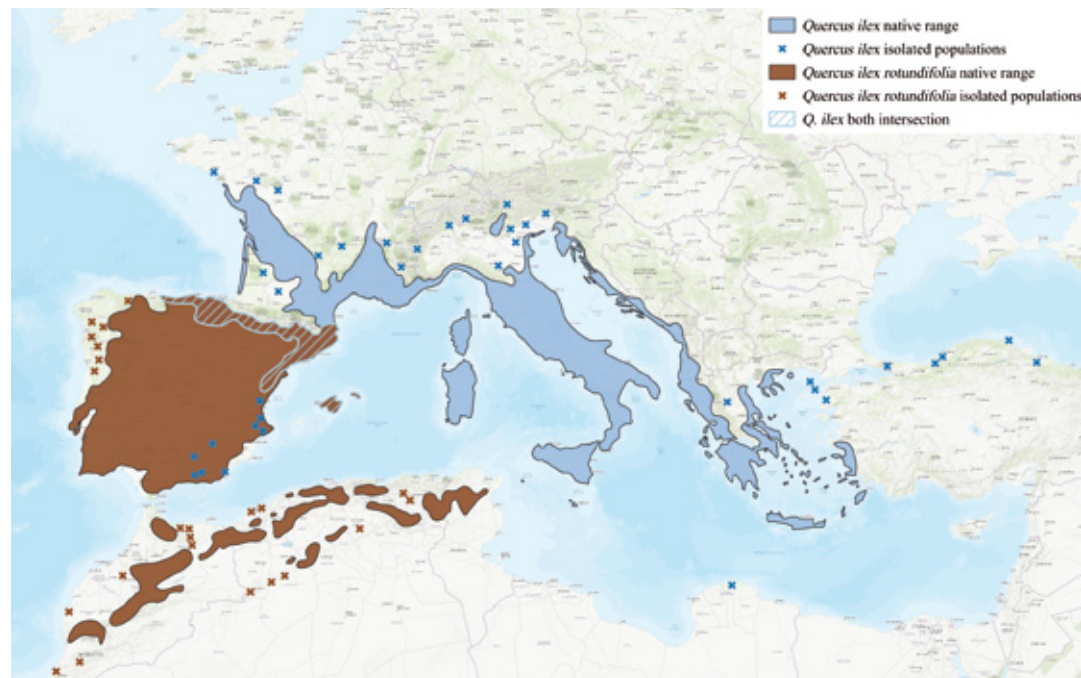


Figure 5.1.3. Holm oak distribution range (EUFORGEN 2009, www.euforgen.org).

Table 5.1.1. List of the sites for *Quercus* spp. of the LIFE SySTEMiC project.

Id	Site name	Country	Species	EFT*	Structure	Silvicultural system
10	Culatta	Italy	<i>Q. robur</i>	5.1	Uneven-aged/ Unmanaged	Unmanaged
13	Nova Gradiška	Croatia	<i>Q. robur</i>	5.1	Even-aged	Uniform shelterwood
20	Pula	Croatia	<i>Q. ilex</i>	9.1	Even-aged	Uniform shelterwood
21	Črni Kal	Slovenia	<i>Q. pubescens</i>	8.1	Even-aged	Irregular shelterwood
28A	Krakovo (Managed)	Slovenia	<i>Q. robur</i>	5.1	Even-aged	Uniform shelterwood
28B	Krakovo (Reserve)	Slovenia	<i>Q. robur</i>	5.1	Uneven-aged/ Unmanaged	Unmanaged

* EFT = European Forest Type: 5.1 Pedunculata oak-hornbeam forest; 8.1 Downy oak forest; 9.1 Mediterranean evergreen oak forest.

5.2 Forest structure deadwood and tree-related microhabitats

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Forest structure, deadwood and tree-related microhabitats were inventoried in six sites (Figure 5.2.1).

In each site, a permanent plot with a representative structure of forest management was chosen. The plot had a rectangular shape and each side was 50 m long (area of 2500 m²). Only when the number of trees of the target species (*Quercus* spp. with diameter at breast height > 2.5 cm) was < 30 trees one more plot was established in the site, until the minimum number of 30 trees was reached.

Detailed protocols for field survey and data elaboration are available on LIFE SySTEMiC web page: <https://www.lifesystemic.eu/>.



Figure 5.2.1. Sites of *Quercus* spp.

Quercus robur L. was the prevailing tree species (basal area > 75%) in Site 28A - Krakovo (Managed), mixed with *Carpinus betulus* L. In the sites 10 - Culatta, 13 - Nova Gradiška and 28B - Krakovo (Reserve) *Quercus robur* L. was most often mixed with *Fraxinus angustifolia* Vahl. (Site 10), *Carpinus betulus* L. (sites 13 and 28A) and other secondary tree species.

Quercus pubescens Willd. was the prevailing tree species in Site 21 - Črni kal, mixed with *Fraxinus ornus* L. and other secondary tree species.

Quercus ilex L. was the most frequent species in Site 20 - Pula, mixed with *Quercus pubescens* Willd. and *Laurus nobilis* L.

The spatial distribution of the trees in the sites is reported in Figure 5.2.2. Figure 5.2.3 shows the stem number-diameter distribution in each site.

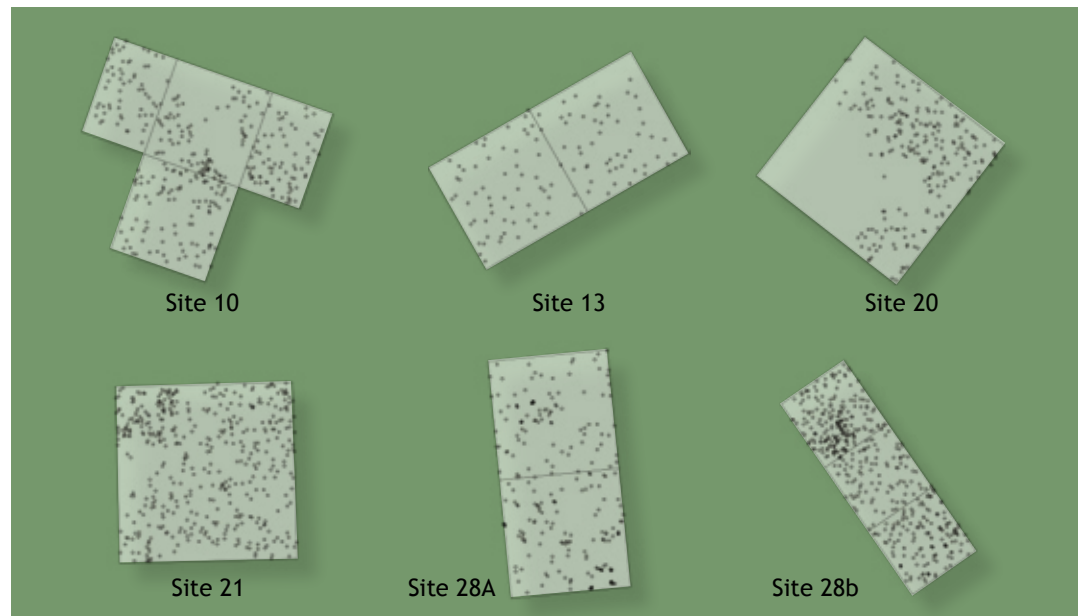


Figure 5.2.2. Spatial distribution of the trees in the sites.

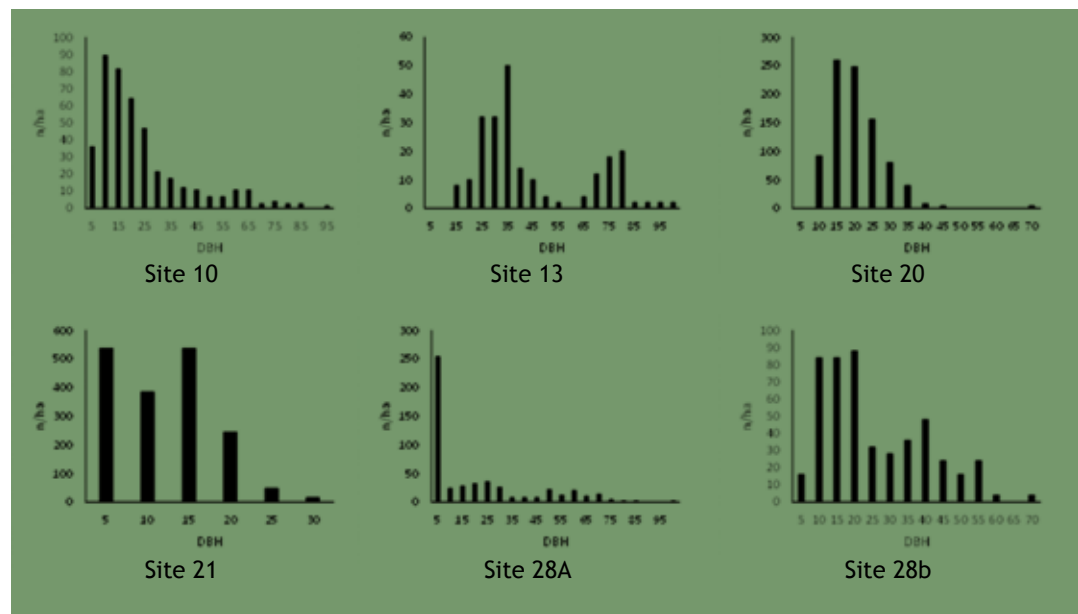


Figure 5.2.3. Stem number-diameter distribution in the sites.

Forest structure variables are reported in Table 5.2.1.

Table 5.2.1. Forest structure variables in the sites. N=number of stems; BA=basal area; V=volume; QMD=quadratic mean diameter (diameter of the mean basal area tree); MH= mean height (height of the mean basal area tree); DH= dominant height (mean height of the 100 trees per hectare with the largest diameters in case of even-aged stand, and mean height of the three tallest trees per hectare in case of uneven-aged stand); SDDBH= standard deviation of DBH; SDH= standard deviation of total tree heights; CVDBH= coefficient of variation of DBH; CVH= coefficient of variation of total tree heights.

Id	N	BA	V	QMD	MH	DH	SDDBH	SDH	CVDBH	CVH
	n/ha	m ² /ha	m ³ /ha	cm	m	m	cm	m	%	%
10	427	29.0	370.7	29.4	22.8	38.2	17.7	8.1	75.0	47.3
13	224	42.8	805.1	49.0	31.3	33.8	21.9	6.7	49.6	23.4
20	892	33.5	274.3	21.9			7.3	1.7	35.5	12.1
21	1764	23.9	93.4	13.1	7.4	9.8	6.1	2.2	52.5	32.8
28A	512	35.6	542.3	29.8	27.2	38.0	21.8	13.5	107.2	84.4
28B	611	41.7	641.9	29.5	22.9	42.6	22.8	9.7	122.1	69.8

In the examined *Quercus robur* L. stands, the basal area ranged between 29 m²/ha and 43 m²/ha and the volume varied between 371 m³/ha and 805 m³/ha. In managed even-aged stands (Sites 13 - Nova Gradiška, 28A - Krakovo (Managed)), the basal area was 40 m²/ha and the volume was 674 m³/ha (average values). The unmanaged stands (Sites 01 - Pian degli Ontani, 28B - Krakovo (Reserve)) had uneven-aged structures, the basal area was 35 m²/ha and the volume was 506 m³/ha (average values).

Forest structure diversity, represented by the tree dimensional diversity (standard deviation and coefficient of variation of DBH and heights), was higher in the unmanaged stands (CVDBH = 98.6%, CVH = 58.6%) than in managed even-aged stands (CVDBH = 78.4%, CVH = 53.9%).

The basal area and the volume in the *Quercus pubescens* Willd. stand (Site 21 - Črni Kal) and in the *Quercus ilex* L. stand (Site 20 - Pula) were 24 m²/ha, 93 m³/ha, 34 m²/ha and 274 m³/ha, respectively.

For each site, the amount of deadwood is reported in Table 5.2.2. In the *Quercus robur* L. stands, the total volume of deadwood ranged between 13 m³/ha and 490 m³/ha. In managed even-aged stands (sites 13 - Nova Gradiška and 28A - Krakovo (Managed)), the total volume of deadwood was 18 m³/ha (average value), which was mostly represented by snags (50%), other lying deadwood pieces (27%) and stumps (20%). The unmanaged stands (Sites 01 - Pian degli Ontani, 28B - Nova Gradiška) had the largest amount of deadwood (272 m³/ha, average value), represented by downed dead trees (49%), snags (36%) and other lying deadwood pieces (14%).

The total amount of deadwood in the *Quercus pubescens* Willd. stand (Site 21) and in the *Quercus ilex* L. stand (Site 20) was 7 m³/ha and 16 m³/ha, respectively.

Table 5.2.2. Volume of deadwood in the sites.

Id	Standing dead trees (including snags)	Downed dead trees	Other lying dead wood pieces	Stumps	Total
	m ³ /ha	m ³ /ha	m ³ /ha	m ³ /ha	m ³ /ha
10	7.5	11.2	34.3	1.6	54.6
13	13.3	0.5	4.8	4.1	22.7
20	6.8	5.4	0.8	2.8	15.8
21	4.1	2.3	0.1	0.2	6.7
28A	4.7	0.5	5.1	3.0	13.4
28B	190.4	257.0	42.3	0.2	489.9

The abundance of the tree-related microhabitats in each site, represented as the percentage of forms of microhabitats, is reported in Figure 5.2.4. As can be seen from the Figure, in the examined sites almost all forms of microhabitats were detected.

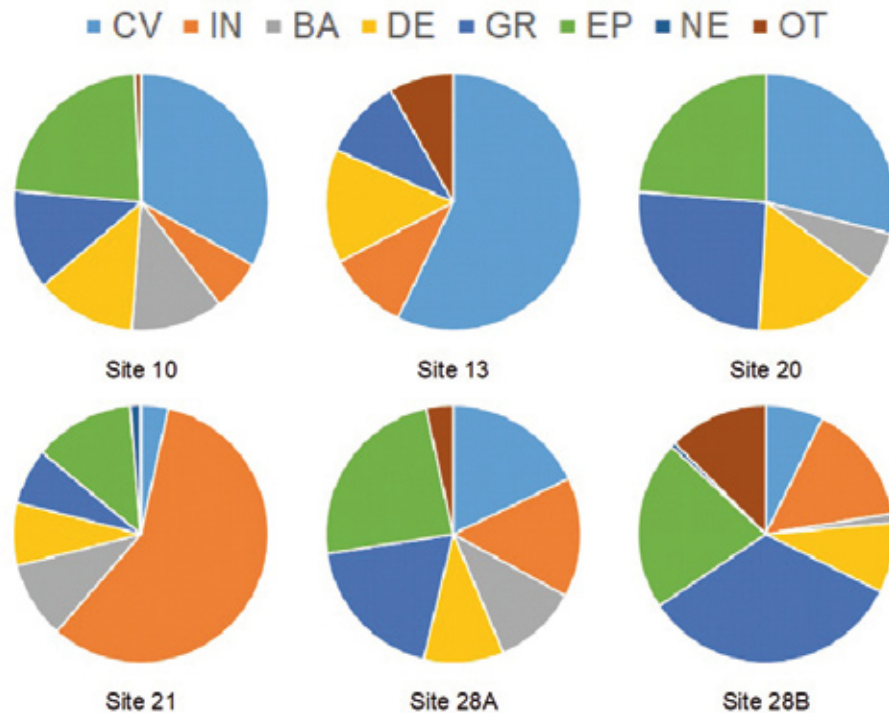


Figure 5.2.4. Tree-related microhabitats in the sites (percentage of forms of microhabitats) (CV = Cavities; IN = Injuries and wounds; BA = Bark; DE = Deadwood; GR = Deformation / growth form; EP = Epiphytes; NE = Nests; OT = Other).



Figure 5.2.5. Deadwood in the Site 10 - Culatta.



Figure 5.2.6. Tree-related microhabitats in the Site 10 - Culatta.

5.3 Landscape genomics

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Genetic variation is crucial for the fitness and survival of individuals within a population, and it is directly correlated with species' ability to adapt to different environments (Balkenhol *et al.*, 2016). Forests, being the most affected ecosystems by human activities, are particularly vulnerable to the erosion of genetic diversity and increased divergence between gene pools, ultimately impacting species survival (Wade *et al.*, 2003; Degen *et al.*, 2021). However, trees, with their high genetic diversity, effective gene flow, and long-life cycles, can mitigate the effects of habitat fragmentation (Kramer *et al.*, 2008; Degen *et al.*, 2021). Understanding the dynamics and mechanisms of local adaptation in natural populations provides basis for predicting responses to environmental changes, including those associated with global climate change. Local adaptation is expected to alter the allele frequencies of genes that influence fitness in particular environments (Lefèvre *et al.*, 2014).

Quercus robur L., being a key component of European broad-leaved forests, faces various challenges due to habitat fragmentation, climate change, and other stress factors (Ducousso and Bordacs, 2004; Degen *et al.*, 2021). In recent decades, pedunculate oak forests in Europe have experienced numerous challenges, including low underground water levels, drought stress, and the effects of climate change, leading to a decline in health status, growth rate, and natural regeneration (Kecic *et al.*, 2021; Degen *et al.*, 2021). The study of the genetic diversity of pedunculate oak populations is essential for understanding their responses to habitat fragmentation, climate change, and other environmental challenges. This knowledge is crucial for designing effective conservation strategies and ensuring the long-term survival of this important forest tree species (Kecic *et al.*, 2021). In this context, landscape genomics has emerged as a flexible analytical framework for understanding the interactions between environmental heterogeneity and adaptive genetic variation in natural populations (Balkenhol *et al.*, 2016). We used Landscape Genomics approaches to analyse the neutral and adaptive component of genetic diversity to highlight possible patterns of local adaptation in the populations analysed. Therefore, we correlated adaptive molecular markers (SNPs) with bioclimatic indicators. Nuclear microsatellite markers (nSSR) were analysed as a measure of neutral genetic variation and structure of the studied populations. Single Nucleotide Polymorphism (SNPs) genotyped using a target re-sequencing approach of candidate genome regions were analysed as a measure of adaptive genetic variation of the studied population (Figure 5.3.1). As a result of *Q. robur* target re-sequencing, about 1600 SNPs were observed in 27 genome regions relevant for response to one or more abiotic stresses (results reported in deliverable Action B1: SNP road-map of each study site). Moreover, as reported in Figure 5.3.1, we have designed road-maps for each target species in order to observe their spatial distribution among all the analyzed population.

The analysed sites related to the target species *Q. robur*, are characterized by two management types: unmanaged forest (Sites 10 - Culatta, 28B - Krakovo (Reserve) and uniform shelterwood system (Site 13 - Nova Gradiška, 28A Krakovo (Managed)). Observing Figure 5.3.1, all sites are characterized by a high number of site-specific SNPs. This could be perceived as an early signature of adaptation to the local environment. The presence of a specific set of national SNPs in the sites located in Slovenia is also interesting. This prevalence of SNPs could be interpreted as a sign of adaptation to a Central Europe/continental bio-climatic regime that characterizes the Slovenian region and sets it apart from the more mediterranean climate found in Italy. As stated in bibliography, analysis for detecting the signature of local adaptation in natural populations requires many analytic steps (Blanquart *et al.*, 2013). The identification of loci with significant correlations with bioclimatic indicators prove to be valuable. For each site, twelve bioclimatic indicators were selected from the existing litera-

ture (Flint *et al.*, 2013; Gugger *et al.*, 2016, 2021; Pluess *et al.*, 2016). To identify local adaptation patterns of the target species, we conducted GEA analyses on two levels: global and site-specific analysis. The global analysis allowed us to identify possible patterns of adaptation to the bioclimatic conditions that characterize the range of oak. The results of the analysis showed the existence of four different genotypes present in Italy, Croatia and Slovenia. Additionally, an even more interesting finding is the presence of association between 42 allelic variants and the mean values of the 12 bioclimatic indicators considered suggest associations that could be interpreted as the basal adaptation genotype of oak across the Central European range. Of particular interest was finding some site-specific allelic variants associated with a particular country (Italy or Slovenia/Croatia) and individual sites. The presence of allelic variants associated with individual sites could be correlated with the local rather than regional pattern of adaptation. In the environmental association analysis (EAA) it is important to account for neutral genetic structure (Rellstab *et al.*, 2015), as neutral genetic structure can produce patterns similar to those expected under non-neutral processes (Excoffier and Ray, 2008; Excoffier *et al.*, 2009; Sillanpää, 2011). Furthermore, the genetic structure of populations was analysed using STRUCTURE (Pritchard *et al.*, 2000) and GENELAND software (Guillot 2008). Observing each site separately we had observed quite interesting results related to the number of SNPs found in association with one or more bioclimatic indicators. We found the highest number of allelic variants (21 SNPs) in unmanaged sites (Site 10 - Culatta; Figure 5.3.2). The presence of a high number of SNPs associated with adaptation to bioclimatic indicators at these sites could be correlated with the neutral genetic structure observed for these sites (Aravanopoulos, 2018; Paffetti *et al.*, 2012; Stiers *et al.*, 2018). Indeed, some studies have observed that these sites are characterized by a complex neutral genetic structure, characterized by a high number of family clusters. This great variability may allow a higher probability for the appearance of new allelic variants that could enrich the adaptive potential of this species for current and future climate change.

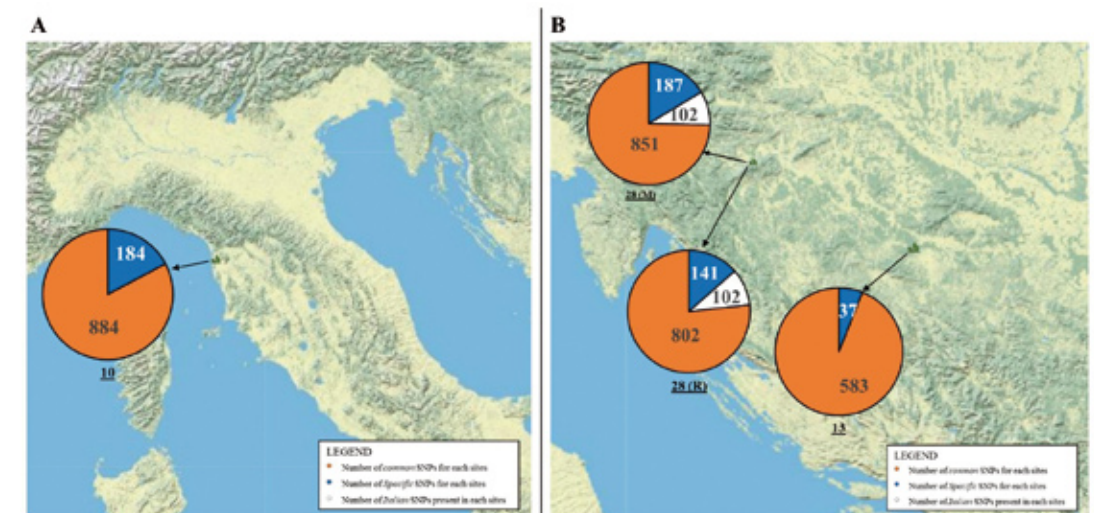


Figure 5.3.1. The road-map of *Quercus robur* L. SNPs distribution in LIFE SySTEMiC sites of Italy (A) and Croatia-Slovenia (B) is depicted in the figure. The figure shows the number of certain specific subsets of SNPs for each site. The data types are sorted by colour. The colours represent the following SNPs subset: The set of SNPs present in that site which are common to at least two sites of interest (orange); the number of unique SNPs specific to that site (blue) and the number of SNPs present in that site, which are specific to the country of origin (white). Below each graph, the identifying number of the corresponding site is displayed (in bold and underlined).

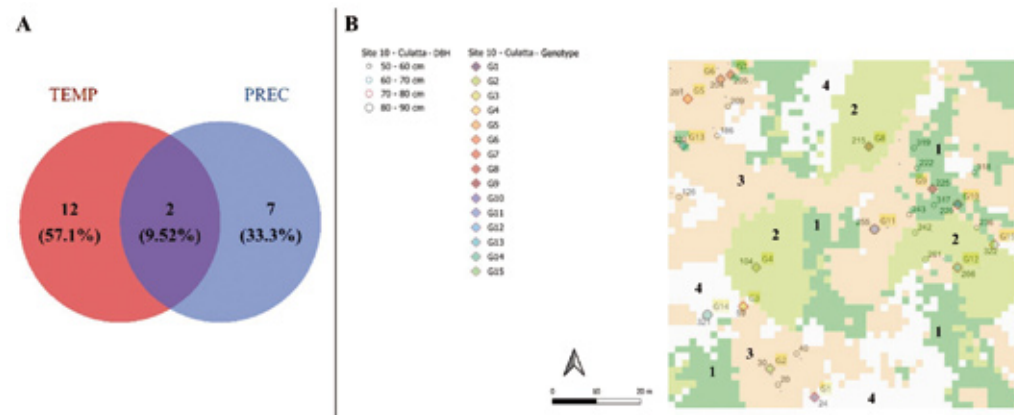


Figure 5.3.2. LFMM analysis results and genotype distribution map of Site 10 - Culatta. (A) Venn diagram showed the overlapping between SNPs associated with temperature-related and precipitation-related bioclimatic indicators, as a result of LFMM analysis. (B) Spatial distribution of genotype and spatial organization in 4 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter is characterized by coloured circles according to the genotype observed. Identical colours mean identical genotypes.

A similar situation was observed for Site 13 - Nova Gradiška (Figure 5.3.3). In this unmanaged population, we had observed a complex spatial genetic structure (simplified in respect to Site 10 - Culatta) with the lowest number of SNPs associated with bioclimatic indicators (9 SNPs). This low number of associated SNPs could be explained also by the absence of site-specific SNPs and by the lowest number of national-specific SNPs reported.

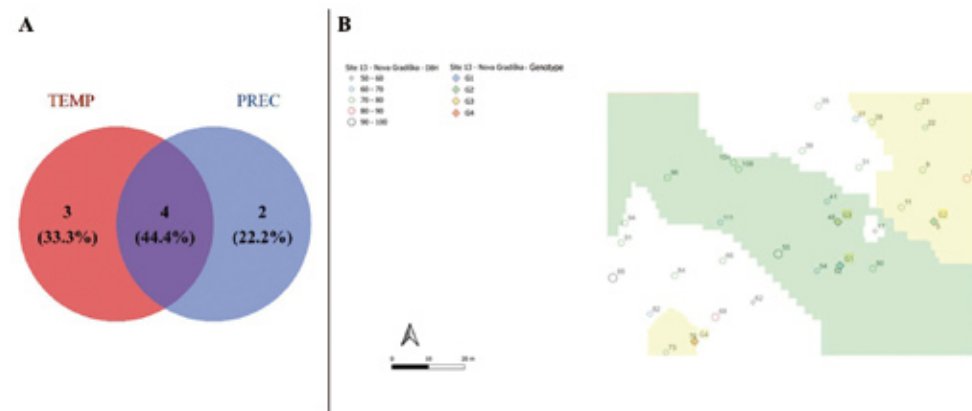


Figure 5.3.3. LFMM analysis results and genotype distribution map of Site 13 - Nova Gradiška. (A) Venn diagram showed the overlapping between SNPs associated with temperature-related and precipitation-related bioclimatic indicators, as a result of LFMM analysis. (B) Spatial distribution of genotype and spatial organization in clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter is characterized by coloured circles according to the genotype observed. Identical colours mean identical genotypes.

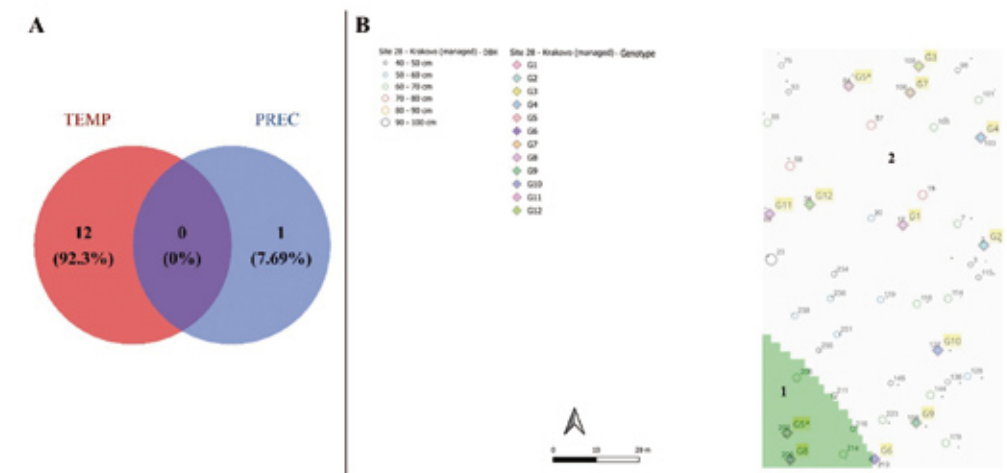


Figure 5.3.4. LFMM analysis results and genotype distribution map of Site 28A - Krakovo (managed) data. (A) Venn diagram showed the overlapping between SNPs associated with temperature-related and precipitation-related bioclimatic indicators, as a result of LFMM analysis. (B) Spatial distribution of genotype and spatial organization in 2 clusters (GENELAND results). The map displays the individuals present within the study site (circle with black border) and the sequenced individuals. The latter is characterized by coloured circles according to the genotype observed. Identical colours mean identical genotypes.

High tree management for timber production is carried out on oak forests in France, some Balkan countries and Germany. In Italy, high-tree forests are targeted for protected areas. In France and Slovenia, the most common type of treatment is uniform and/or irregular shelterwood system. English oak is a heliophilous and low competitive species that prospers in disadvantageous environments where competitive species are less active. The dynamics of English oak renewal are related to the presence of dense subshrub. The absence of consociated species renovation favours English oak renewal.

Less impactful management types, such as individual tree selection, appear to report population with a high number of allelic variants associated with response to bioclimatic indicators. Similar results have been observed in unmanaged stands and old-growth forests. The results reported in this study could be important in silvicultural management planning, where knowledge of genetic variability from an adaptive perspective could help decision-making processes. In addition, this knowledge could also be used in anticipation of assisted migration works. This is important to preserve the current Forest Genetics Resources (FGR), but also to enrich the existing stand with potentially favourable genotypes.

5.4 Oak mildew

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Pedunculate oak (*Quercus robur* L.), a keystone tree species in Europe, faces an uncertain future in (near-) natural forests due to challenges in natural regeneration. One of the primary factors contributing to this uncertainty is a fungal disease known as oak mildew (*Erysiphe alphitoides* complex). The fungi significantly affect the shade tolerance and vertical growth of seedlings and saplings, leading to a marked reduction in their vitality and competitiveness. As a result, natural regeneration under the oak canopy is often hindered by mildew infections (Demeter *et al.*, 2021).

When oak powdery mildew infects immature leaves, the powdery coating spreads across the entire leaf surface, leading to uneven development or potential drying. This proliferation is particularly detrimental to saplings, limiting their growth and causing significant mortality. In contrast, the powdery coating on adult leaves remains localized, resulting in moderate damage to mature trees (Thomas *et al.*, 2002; Marçais and Breda, 2006). The fungi produce spores (conidia) that are easily dispersed by wind, insects, and splashing water. These spores germinate and infect new plant tissues, especially under conditions of high humidity and moderate temperatures.

As part of the LIFE SySTEMiC project different ways of controlling oak mildew at one of our experimental sites in Krakovo Forest were tested. Krakovo Forest is the largest lowland floodplain forest in Slovenia, dominated by pedunculate oak. The presence of powdery oak mildew is so extensive that it represents one of the limiting factors of natural regeneration.



Figure 5.4.1. Pedunculate oak seedling with an oak mildew infection.

The objective of the study was to assess the impact of planting density and varying concentrations of AQ-10 biopesticide on powdery mildew infection in seedlings. The experiment involved planting in a fenced area, following the 'Protocol for planting experiment: oak powdery mildew control protocol' (Figure 5.4.2, left). Planting density effects were examined using two densities: approximately 1100 and 4400 seedlings per hectare. Biological control effects were evaluated with two concentrations of AQ-10: half the recommended concentration, the full recommended concentration, and a control group with no treatment.

In all treatment types, we assessed mortality rates and height growth, while infection rates were evaluated visually using a 5-point scale. Our results did not show differences between the various treatments, neither in height growth nor in mortality, which averaged between 29.2% and 31.9% across all treatments after two years. Infection intensity proved to be an inappropriate measure in our case because, at the beginning of the growing season during the biocontrol spraying, a large proportion of the leaf area was already damaged or missing due to defoliators (Figure 5.4.2, right), which hindered reliable assessment. Later

in the growing season, 'lammas' growths (second and third flush in mid-summer) replaced most of the leaf area that had developed in spring, leaving the newly formed leaf area untreated. Based on our results, the biofungicide AQ-10 did not exhibit the desired effects against oak mildew, and planting density had no impact.

Despite our discouraging results, it remains crucial to explore various methods for protecting against oak mildew, which are primarily implemented in forest nurseries. Currently, several methods are available to combat the disease, such as conventional fungicides like those based on citric acid, antagonistic bacteria or fungi, and commercial chemical treatments such as sulphur, potassium bicarbonate, potassium phosphite, and salicylic acid, along with efforts to develop genetic resistance (Turczanski *et al.*, 2023).

Effective control of powdery mildew on pedunculate oak seedlings and young oak plants is vital for regeneration areas during regeneration cutting phases. Well-established one- and two-year-old oak plants tolerate powdery mildew well, and the fungus no longer hinders their growth (Pap *et al.*, 2012).

Conservation efforts and forest management plans should account for the impact of mildew to enhance natural regeneration, promote close-to-nature management of pedunculate oak forests, and support associated biodiversity. Canopy opening is recommended to facilitate further development of saplings (Demeter *et al.*, 2021). Shelterwood cutting is recognized as one of the most effective and reliable methods for natural regeneration of pedunculate oak (Dobrowolska, 2008).



Figure 5.4.2. Experimental plot in Krakovo forest (left) and gypsy moth larva (*Lymantria dispar* L.).

5.5 GenBioSilvi model

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Measuring genetic diversity is crucial to understand the status of forest ecosystems as well as supporting conservation and management efforts.

Based on the results obtained, we can assume that there is high genetic diversity in all sites but we cannot conclude that there is significant genetic diversity between the sites even though they differ in management types. Regarding *Q. robur* sites, we studied two unmanaged forests and two sites managed with a uniform shelterwood system. High tree management for timber production is carried out on oak forests in France, some Balkan countries and Germany. In Italy, high-tree forests are targeted for protected areas. In France and Slovenia, the most common type of treatment is uniform and/or irregular shelterwood system.

Since we considered only unmanaged sites and only two treated sites, we do not have sufficient data to be able to determine which type of management promotes genetic diversity.

Analysing the pattern of genetic diversity distribution based on SSR data, we observed that Site 10 - Culatta (unmanaged) and Site 13 - Nova Gradiška (uniform shelterwood system), have a complex and heterogeneous spatial genetic structure. This can be attributed to the non-random mating between closely related individuals. From the results obtained for each studied stand, it was possible to observe a simplified spatial genetic structure in the other sites.

The complexity reported for Site 10 and Site 13 implies a dynamic and adaptable ecosystem capable of responding to environmental changes by generating new genetic variability through recombination and gene flow between subpopulations. In addition, we observed a high number of SNPs correlated with current environmental conditions across sites. The presence of these allelic variants associated with bioclimatic indicators that best characterize the local environment is important. We found that Site 10 - Culatta (unmanaged) presented a higher number of SNPs correlated with bioclimatic indicators.

Stand structure is a key element in assessing ecological functions and services in forest ecosystems. To describe biodiversity, we observed dendrometric data to define the forest structure of the analysed sites. Thanks to this data, we can assume that the best structure observed is that characteristic of a multi-layered uneven-aged forest, considering all the species present. When we analysed only *Q. robur* individuals in all sites, we observed a bell-shaped mono-stratified structure, typical of English oak stands. A multi-layered uneven-aged population supports the presence of natural regeneration because it shows gaps in canopy cover that create ideal conditions for the establishment of natural regeneration of competitive species. In addition, multi-layered forest allows high pollen dispersal, promoting genetic diversity. Therefore, based on the best parameters used to define forest structure, we observed that sites characterized by multilayered uneven-aged forest structure (considering all species in stands) also exhibit complex spatial genetic structure and high genetic diversity.

Deadwood plays crucial functional roles such as in nutrient cycling and as a carbon sink. The presence of deadwood can promote germination and seedling development of tree species, acting as nursery sites and thus contributing to forest renewal.

Regarding the amount of deadwood, the best situation is observed in unmanaged sites Site 28B - Karakovo reserve that showed a higher volume of deadwood.

Deadwood provides an important microhabitat for the development and conservation of species important to the forest ecosystem. In addition to deadwood, we observed other indicators related to saproxylic microhabitats. The greatest number of microhabitats was detected at sites where there are century-old individuals with stem deformations and cavities. The frequency of epixylic microhabitats is lower than saproxylic in all observed populations. Epixylic are used as indicators to assess ecosystem conditions. The presence of these microhabitats is an important source of biodiversity. Based on the obtained results, management types that allow for complex forest structures characteristic of uneven-aged and multilayered stands increase the probability of observing different microhabitat forms.

Based on the results obtained during the analyses, through the observation of these indicators it is possible to simulate the genetic diversity status of the stand and guide users toward sustainable management to conserve and/or increase the genetic diversity present.

Considering the results obtained from the analysis of all biodiversity indicators, we developed a model that describe the current status of genetic resources within the site. This model was developed to help forest users for checking the current status of stand biodiversity and providing guidelines for sustainable management. To develop a user-friendly suitable model, we observed that it is possible to identify a set of key indicators that are more representative. The indicators at the basis of the model can indirectly describe the genetic diversity status of the stand, as seen from the results obtained. In addition, it is also possible to identify some indicators that are more representative of biodiversity in terms of deadwood, microhabitat, and species diversity. For this reason, we decided to exclude in the form developed for users the data collection on genetic diversity and soil diversity that are difficult to observe.

In this regard, we have developed a model that involves the observation of some key indicators that describe the current status of the analyzed stand. The selected indicators are shown in Table 5.5.1.

Table 5.5.1. Description of selected indicator useful for users to describe the status of the stand.

Categories	Indicators	Description
Forest structure	DBH standard deviation	Variability in tree DBH within the stand
	Percentage of trees in regeneration layer	Presence of the target species natural regeneration
	DBH class distribution curve	Complexity of horizontal and vertical forest structure
	No. of population strata	
Deadwood	Standing deadwood presence	Description the presence of deadwood functioning as microhabitat
	Coarse woody debris	
Species diversity	Species richness	Number of all species present in the stand regarding both the presence of adults' individuals and regeneration
	Percentage of non-target individuals regeneration	
Microhabitat	Percentage of individuals with cavities	Presence of key microhabitat form for biodiversity
	Percentage of individuals with injuries and wounds	
	Percentage of individuals with deformation	

The model involves the production of a form that requires the compilation of certain parameters that can be easily observed in the field by users. Based on our results, we have chosen value thresholds for each selected indicator. The annotation of the actual real data for each indicator will produce a score based on the selected thresholds. The final score is associated with the identification of the current status of the stand analysed. For each final score, indications are provided regarding possible actions to be taken to implement sustainable management of the stand (Fig. 5.5.1).

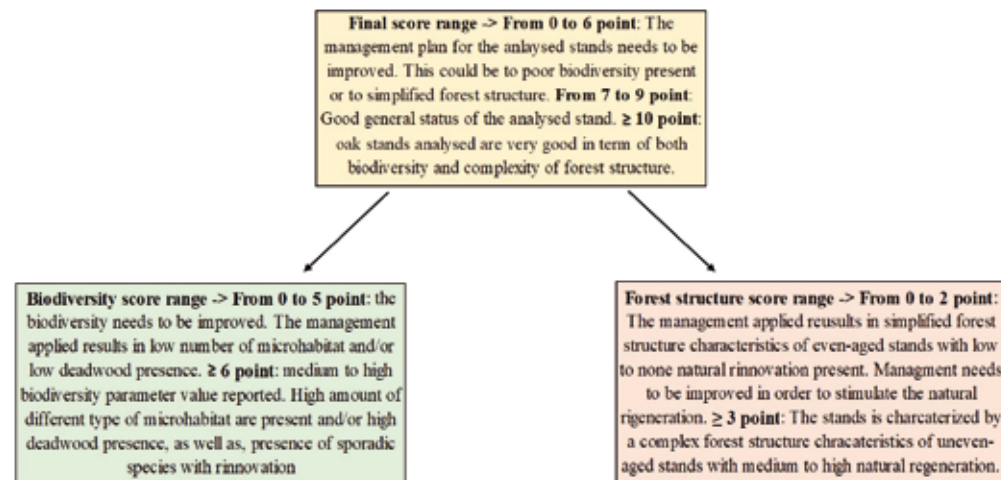


Figure 5.5.1. Guideline for forest management based on the final score obtained after forest assessment forest form compilation.

Forest stand evaluation form			
Forest management	Real Data		Implication for Management
Forests should be managed in a way to preserve their multifunctional role (ecological, social and productive forest functions). This can be achieved only through maintenance of healthy forests and their biodiversity, protection of its natural fertility and water sources as well as other beneficial functions of forests in the water and carbon cycle, sustainable supply of wood and other products from forest, profit and employment.			
Forest structure indicator	Real Data	Indicator-specific score	Implication for Horizontal and Vertical Forest structure
Structural indicator			
Forest structure is both a product and driver of ecosystem processes and biological diversity. Changes in forest structure as a result of management for timber production have undesirable consequences for other components of forest ecosystems	DBH Standard deviation	<input checked="" type="checkbox"/> Score 1 (DBH SD < 10) <input type="checkbox"/> Score 2 (10 ≤ DBH SD ≤ 20) <input type="checkbox"/> Score 3 (DBH SD > 20)	Results from annotated parameters <input type="checkbox"/> Score 1 (Single forest structure without rinnovation) - From 4 to 6 <input type="checkbox"/> Score 2 (Simplified forest structure with rinnovation or Complex forest structure without rinnovation) - From 7 to 8 <input type="checkbox"/> Score 3 (Complex forest structure with rinnovation) - From 9 to 11 <input type="checkbox"/> Score 4 (Complex forest structure with rinnovation present in the total area) - From 12 to 13
	Refers to % of trees in regeneration layer (considering DBH less than 10 cm)	<input checked="" type="checkbox"/> Score 1 (% Rim < 15) <input type="checkbox"/> Score 2 (15 ≤ % Rim < 30) <input type="checkbox"/> Score 3 (30 ≤ % Rim < 50) <input type="checkbox"/> Score 4 (% Rim ≥ 50)	
	Curve of DBH class distribution	<input type="checkbox"/> Score 1 (Bell-shaped) <input type="checkbox"/> Score 2 (Multi-modal) <input type="checkbox"/> Score 3 (J-shaped)	
	N° of strata (stratification of population)	<input type="checkbox"/> Score 1 (Mono-stratified) <input type="checkbox"/> Score 2 (Bi-stratified) <input type="checkbox"/> Score 3 (Tri or Multi-stratified)	
Productivity indicators	Real data		Implication for population biodiversity
Forest site productivity is the production that can be realized at a certain site with a given genotype and a specified management regime. Site productivity depends both on natural factors inherent to the site and on management-related factors.	Growing stock (m³/ha)		In managed stands, the minimum growing stock should be around 250-350 m³/ha.
Biodiversity indicators	Real data	Indicator-specific score	Implication for stand biodiversity
Deadwood indicators	Standing deadwood (including snags) presence	<input type="checkbox"/> Score 1 (Absence of standing deadwood) <input type="checkbox"/> Score 2 (Presence of standing deadwood)	Results from annotated parameters <input type="checkbox"/> Score 1 (Total absence of deadwood) - 2 point <input type="checkbox"/> Score 2 (Presence of standing deadwood or Coarse woody debris) - 3 point <input type="checkbox"/> Score 3 (Presence of various type of deadwood) - 4 point
		<input type="checkbox"/> Score 1 (Absence of Coarse woody debris) <input type="checkbox"/> Score 2 (Presence of Coarse woody debris)	
Species diversity indicators	Species richness (presence of individual of non target species)	<input type="checkbox"/> Score 1 (Absence of other species) <input type="checkbox"/> Score 2 (Presence of other species)	Results from annotated parameters <input type="checkbox"/> Score 1 (Monospecific site) - 2 point <input type="checkbox"/> Score 2 (Sporadic species with none or low regeneration) - 3 point <input type="checkbox"/> Score 3 (Sporadic species with high regeneration) - 4 to 6 point
		<input checked="" type="checkbox"/> Score 1 (% Rim < 15) <input type="checkbox"/> Score 2 (15 ≤ % Rim < 30) <input type="checkbox"/> Score 3 (30 ≤ % Rim < 50) <input type="checkbox"/> Score 4 (% Rim ≥ 50)	
% of non-target individuals in the smallest diameter class (10 cm)		<input type="checkbox"/> Score 1 (Absence of other species) <input type="checkbox"/> Score 2 (Presence of other species)	Results from annotated parameters <input type="checkbox"/> Score 1 (Monospecific site) - 2 point <input type="checkbox"/> Score 2 (Sporadic species with none or low regeneration) - 3 point <input type="checkbox"/> Score 3 (Sporadic species with high regeneration) - 4 to 6 point
		<input checked="" type="checkbox"/> Score 1 (% Rim < 15) <input type="checkbox"/> Score 2 (15 ≤ % Rim < 30) <input type="checkbox"/> Score 3 (30 ≤ % Rim < 50) <input type="checkbox"/> Score 4 (% Rim ≥ 50)	
Microhabitat indicators	Real data	Indicator-specific score	Implication for stand biodiversity

% of individuals with cavities	<input checked="" type="checkbox"/> Score 1 (% Ind. ≤ 15%) <input type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%) <input type="checkbox"/> Score 3 (% Ind. > 50%)	Results from annotated parameters <input checked="" type="checkbox"/> Score 1 (Absence of low number of microhabitat) - 3 point <input type="checkbox"/> Score 2 (Sporadic to consistent presence of microhabita) - 4 to 6 point <input type="checkbox"/> Score 3 (High presence of microhabitat) - 7 to 9 point
% of individuals with injuries and wound	<input checked="" type="checkbox"/> Score 1 (% Ind. ≤ 15%) <input type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%) <input type="checkbox"/> Score 3 (% Ind. > 50%)	
% of individuals with deformation	<input checked="" type="checkbox"/> Score 1 (% Ind. ≤ 15%) <input type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%) <input type="checkbox"/> Score 3 (% Ind. > 50%)	
Final Score: 1		

Figure 5.5.2. Forest population assessment form structure.

Forest population assessment sheet - Simulation Site 10 (Culatta)			
Forest management	Real Data	Implication for Management	
Forests should be managed in a way to preserve their multifunctional role (ecological, social and productive forest functions). This can be achieved only through maintenance of healthy forests and their biodiversity, protection of its natural fertility and water sources as well as other beneficial functions of forests in the water and carbon cycle, sustainable supply of wood and other products from forest, profit and employment.	Unmanaged		
Forest structure indicator	Real Data	Indicator-specific score	Implication for Horizontal and Vertical Forest structure
Structural indicator DBH Standar deviation Refers to % of trees in regeneration layer (considering DBH less than 10 cm) Curve of DBH class distribution N° of strata (stratification of population)	14,31 0 Bell-shaped Bi-stratified	<input type="checkbox"/> Score 1 (DBH SD < 10) <input checked="" type="checkbox"/> Score 2 (10 ≤ DBH SD ≤ 20) <input type="checkbox"/> Score 3 (DBH SD > 20) <input checked="" type="checkbox"/> Score 1 (% Rinn < 15) <input type="checkbox"/> Score 2 (15 ≤ % Rinn < 30) <input type="checkbox"/> Score 3 (30 ≤ % Rinn < 50) <input type="checkbox"/> Score 4 (% Rinn ≥ 50) <input checked="" type="checkbox"/> Score 1 (Bell-shaped) <input type="checkbox"/> Score 2 (Multi-modal) <input type="checkbox"/> Score 3 (J-shaped) <input type="checkbox"/> Score 1 (Mono-stratified) <input checked="" type="checkbox"/> Score 2 (Bi-stratified) <input type="checkbox"/> Score 3 (Tri or Multi-stratified)	Results from annotated parameters <input checked="" type="checkbox"/> Score 1 (Simplified forest structure without rinnovation) - From 4 to 6 <input type="checkbox"/> Score 2 (Simplified forest structure with rinnovation or Complex forest structure without rinnovation) - From 7 to 8 <input type="checkbox"/> Score 3 (Complex forest structure with rinnovation) - From 9 to 11 <input type="checkbox"/> Score 4 (Complex forest structure with rinnovation present in the total area) - From 12 to 13
Productivity indicators	Real data	Implication for population biodiversity	
Forest site productivity is the production that can be realized at a certain site with a given genotype and a specified management regime. Site productivity depends both on natural factors inherent to the site and on management-related factors.	367,5 m ³ /ha	In managed stands, the minimum growing stock should be around 250-350 m ³ /ha.	
Biodiversity indicators	Real data	Indicator-specific score	Implication for stand biodiversity
Deadwood indicators Standing deadwood (including snags) presence Coarse woody debris	Presence Presence	<input type="checkbox"/> Score 1 (Absence of standing deadwood) <input checked="" type="checkbox"/> Score 2 (Presence of standing deadwood) <input type="checkbox"/> Score 1 (Absence of Coarse woody debris) <input checked="" type="checkbox"/> Score 2 (Presence of Coarse woody debris)	Results from annotated parameters <input type="checkbox"/> Score 1 (Total absence of deadwood) - 2 point <input type="checkbox"/> Score 2 (Presence of standing deadwood/ Coarse woody debris) - 3 point <input checked="" type="checkbox"/> Score 3 (Presence of various type of deadwood) - 4 point

Biodiversity conservation is a key objective for sustainable forest management, but the multi-dimensional and multi-scale character of biodiversity renders full assessment difficult at large scale. Therefore, indicators are often used to monitor biodiversity. The quantity and quality of deadwood are commonly used in nature conservation as indicators of forest biodiversity. Additionally, the presence of species different from the target species as well as the presence of specific type of microhabitat are also used to monitor biodiversity in forest stand.	Species richness (presence of individual of non-target species)	Presence	<input type="checkbox"/> Score 1 (Absence of other species) <input checked="" type="checkbox"/> Score 2 (Presence of other species)	Results from annotated parameters <input type="checkbox"/> Score 1 (Monospecific site) - 2 point <input type="checkbox"/> Score 2 (Sporadic species with none or low regeneration) - 3 point <input checked="" type="checkbox"/> Score 3 (Sporadic species with high regeneration) - 4 to 6 point
	% of non-target individuals in the smallest diametric class (10 cm)	38,43	<input type="checkbox"/> Score 1 (% Rinn < 15) <input type="checkbox"/> Score 2 (15 ≤ % Rinn < 30) <input checked="" type="checkbox"/> Score 3 (30 ≤ % Rinn < 50) <input type="checkbox"/> Score 4 (% Rinn ≥ 50)	
	Microhabitat indicators	Real data	Indicator-specific score	Implication for stand biodiversity
	% of individuals with cavities	29,8	<input type="checkbox"/> Score 1 (% Ind. ≤ 15%) <input checked="" type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%) <input type="checkbox"/> Score 3 (% Ind. > 50%)	Results from annotated parameters <input type="checkbox"/> Score 1 (Absence of low number of microhabitat) - 3 point <input checked="" type="checkbox"/> Score 2 (Sporadic to consistent presence of microhabita) - 4 to 6 point <input type="checkbox"/> Score 3 (High presence of microhabitat) - 7 to 9 point
% of individuals with injuries and wound	7,14	<input checked="" type="checkbox"/> Score 1 (% Ind. ≤ 15%) <input type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%) <input type="checkbox"/> Score 3 (% Ind. > 50%)		
% of individuals with deformation	0,62	<input checked="" type="checkbox"/> Score 1 (% Ind. ≤ 15%) <input type="checkbox"/> Score 2 (15% < % Ind. ≤ 50%) <input type="checkbox"/> Score 3 (% Ind. > 50%)		
Final Score: 9				

Figure 5.5.3. Forest population assessment form structure with Site 10 - Culatta data.

Below is the example of the form we produced for compilation (Fig. 5.5.2). Instead, figure 5.5.3 shows the compiled form based on the actual data obtained from the Site 10 - Culatta.

5.6 Recommendations for Sustainable Forest Management

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Overview of silvicultural systems

Among the 13 European white oak species, pedunculate (*Q. robur*) and sessile (*Q. petraea*) oaks are the most important, economically and ecologically, deciduous forest tree species in Europe (Diaci, 2006), while other oak species such as downy oak (*Q. pubescens*) and holm oak (*Q. ilex*) which have been also studied within LIFE SySTEMiC project are gaining their importance due to their resistance to climate change.

Oaks are widely distributed in Europe, from northern Spain to southern Scandinavia and from Ireland to Eastern Europe. Also, oaks are closely related, they can mix, compete and naturally hybridize with one another. Oaks are therefore among the most diverse species of forest trees. High levels of diversity are most likely due to the maintenance of large population sizes, overlapping of ecological niches, long-distance geneflow and their interfertility (Ducousso and Bordacs, 2004). Human impact on oak populations is very large, most oak forests are managed in different ways. Analysed oak stands within LIFE SySTEMiC have been mostly even aged, unmanaged or managed as irregular or uniform shelterwood system. Silvicultural system that is most suitable for pedunculate oak forests is irregular shelterwood with larger openings between 0.5 and 2 ha or uniform shelterwood system, which covers oak demand for light. Silvicultural systems that are rarely used in the area are the intensive management of even aged forests, while the coppice system is common in Italy, especially in downy

oak and holm oak forests (Ciancio and Nocentini, 2004). Additionally an abandonment of any forest management of mediterranean oak stands on steep-slope forests with limited accessibility can also be the case.

Silvicultural characteristics of oaks

Sustainable forest management of studied oak forests (*Q. robur*, *Q. pubescens*, *Q. ilex*) has to be adapted to forest site individual characteristics and to main observed directions of forest development. Oak site characteristics can change in short distances. If we want to preserve these characteristics and benefit from their specifics to the maximum extent, suitable mixed tree and forest stands structures should be used. Directed development of oak stands adapted to individual site and stand conditions, demands great flexibility in selection of a proper system (method) of forest management and careful planning of measures. Oaks species differ morphologically and in terms of their site conditions requirements (Diaci, 2006). All of them are light demanding species, at a young age they grow quickly in height. On better sites with no influence of overstorey trees they reach a culmination of increment already between 30 and 45 years of age. Thereafter, the volume increment decreases but not rapidly so that the average volume increment of 200 years old stands is still almost the highest (Diaci, 2006). Especially in young phases oaks respond perfectly to silvicultural measures which have an important effect on tree form and stand structure.

Threats

Regarding the present threats and risks in oak forests with the increase of average annual temperatures, oak sites will gradually become drier which will lead to the reduction of suitable area for oaks, mostly for *Q. robur* and *Q. petraea* (ZGS, 2021). Due to the complex action of biotic (diseases, pests) and abiotic factors (drought), the proportion of growing stock in older development stages is also partly at risk. With increased aridity, the fire risk of oak forests will also increase. Admixture of conifers with a high proportion of forest decline (red and black pine) also alter the vulnerability of those forests. Key conservation problems in oak forests are fungal diseases (oak mildew) and pests, rapid development of non-native invasive plant species and reduction of the possibility for natural oak rejuvenation. *Q. robur* stands, due to degradation, changes in tree species composition and changes in the temperature and water regime, face a lack of suitable stands for seeding or with the absence of full crops. As a result of the invasion of non-native invasive plant species, soil properties will change and deteriorate. The presence of diseases and pests causes unplanned measures that can significantly disrupt the planned dynamics of oak forest restoration (ZGS, 2021).

The big threat to genetic diversity of the oaks is also the introduction of exotic genotypes through plantations. White oaks have very large ecological niches and sometimes occupy extreme habitats (rocky slopes in mountains, sand dunes, saline soils, peat bog, garigues). These populations are at high risk of disappearing because the number of individuals is low, habitats are unstable and human impact is often considerable (Bajc *et al.*, 2020).

Natural regeneration can also be a problem for oaks. Due to the unbalanced ratio of development phases, over-abundance of game or changes in groundwater regime natural regeneration is limited. In many cases seedlings die a couple of years after germination (ZGS, 2021).

Pests and pathogens also represent a serious threat. Oak mildew (*Microsphaera alphitoides*) is reported to be of the most common oaks pathogen. Acute oak decline is a new syndrome affecting principally pedunculate and sessile oaks, and this has become a more widely recognized problem in recent years. Defoliation of the first flush of leaves is common by several caterpillars, e.g. *Tortrix viridana*, *Lymantria dispar*, *Operophtera brumata* and *Thaumetopoea processionea*, oak processionary moth which has spread from southern Europe further north (Bajc *et al.*, 2020).

Mediterranean oaks are facing additional threat in overharvesting and overgrazing and climate

change, indiscriminate cutting, improper silvicultural management (coppices or clear cuttings over large areas, where regeneration cannot succeed) and intensive ungulate browsing (Bordacs *et al.*, 2019).

Assessment of adaptation potential of oak forests to climate change

The climate change adaptation potential of *Q. robur* forests is quite low due to many threats to pedunculate oak as key species. In *Q. pubescens* and *Q. ilex* forests it is larger since those tree species are more resistant to drought. These forests also consist of tree species that are capable of good regeneration from stumps, which is especially beneficial in the event of various disasters (fires), but such forests are uninteresting for economic exploitation what reduces the actual extent of active management. Despite this, it makes sense to improve their adaptability to climate change, primarily by facilitation of forest stand seed production and the gradual transformation of coppice stands (Bordacs *et al.*, 2019).

Regeneration of oak stands

Regeneration of preserved pedunculate oak stands should be done after the oak seed year. Stands are restored primarily naturally and where necessary also by planting and sowing. When introducing an oak stands for restoration, it is important that the understory tree layer is not completely removed, due to the favourable effect on the microclimate, the protection of the saplings and the prevention of the intensive growth of weeds and shrubs (ZGS, 2021).

Restoration is then followed by a series of two or three felling: preparatory felling with 30% strength, seeding felling with 50% and final felling. Felling has to be done quite quickly with an interval of 3 to 5 years. The understory tree layer should be completely removed within two to three years after seeding.



Figure 5.6.1. Introduction of oak stands into regeneration is done after seed year.

Tending and protection of oak stands

Most suitable tending and protection measures are summarized based on SFM guidelines in Slovenia (ZGS, 2021) and LIFE SySTEMiC results. In *Q. robur* stands we ensure the opportunity for the rapid development of strong crowns of the oaks and thus the stability of the forests against harmful abiotic influences (wind, snow) with proper execution of tending measures (early and heavy thinning).

Intensive care of young trees is necessary to ensure the proper stand structure of the future forest. In natural young growth priority to balancing the mixture of tree species is needed. In the initial phase, the biggest problem is the understory layer, which grows faster than oak stand, so it must be removed annually until the oak is no longer threatened. In the case of artificially regenerated oak, due to the fast growing herbaceous and shrub vegetation, regular and timely tending is necessary, twice a year, depending on the conditions on the ground. If the loss is more than 30%, we carry out additional planting with oak and noble deciduous trees (mountain maple, wild cherry) (ZGS, 2021).

In young growth phase it is essential that oak mildew negative effect is controlled since it represents one of the limiting factors of natural regeneration.



Figure 5.6.2. Regular tending of young growth is needed due to the competition of herbaceous and shrub vegetation.

Tending of young growth is usually carried out once, after the positive qualities of individual young trees are already evident. With selective thinning we must start early. Strong thinning of the oak stands ensures the possibility of rapid development of strong crowns and greater stability. Thinning of oak pole stands is necessary to provide a sufficiently large growing space for the selected trees. Their crowns should be wide enough.

We also have to try to preserve the understory layer, which consists of different admixture species.

In principle, we do not interfere with the layer of understory trees, but if we do, we promote the best quality specimens through selective thinning.

In adult stands, thinning only makes sense in the younger parts of the stands (12-16% of growing stock). Understory in the stand must be protected until the beginning of final harvesting, that is, before the introduction of the stand for restoration.

On poorer productive sites of *Q. pubescens* and *Q. ilex*, mostly no care or minimal care is needed. This should be aimed primarily at oak and pine stands. On more productive sites tending measures in young growth is carried out only once during the young growth period.

Shortening the production period makes sense in order to maintain the vitality of forest stands and to achieve economic effects in stands where the tree composition has changed or in stands where the health of the oak is poor, and the trees are dying.

Adaptation of oak stands to climate change

Genetic resources of oaks are endangered not only by the loss of natural ecosystems and limitation of seed sources but also by the impact of air pollution for several decades and by long-term climate changes (Bajc *et al.*, 2020).

The forest restoration system in oak stands needs to be adapted to the increasingly frequent natural disasters and to determine the priorities for action after natural disasters and methods of restoration of damaged forests.

The size of the areas for restoration must be smaller, as this ensures the mosaic structure of future stands and increases their resilience. Nevertheless, we must not ignore the light/growth requirements of individual tree species and narrow down the species diversity of the future young growth.

The relationship between natural regeneration and regeneration through planting must follow other strategies, especially in terms of ensuring the constant coverage of forest areas and ensuring the genetic diversity of the young forest. Where possible, natural regeneration is used, as this way the evolutionary process remains less disturbed. The problem arises when the environment changes faster than the trees can adapt. This can lead to reduced vitality and even to a critical point where the population can no longer regenerate itself.

The main orientation still remains rejuvenation under the canopy and indirect care with the help of the mature stand. Rejuvenation periods need to be critically re-evaluated and shortened wherever possible or extended in certain forest stands.

With appropriate, sufficiently frequent and sufficiently intensive tending of oak stands, we can influence on the improvement of the structure of stands and thus reduce susceptibility to natural disturbances and reduce the impact of negative biotic and abiotic factors. It is also necessary to adjust (mainly reduce) the density of forest stands and ensure greater heterogeneity of stand structure (more vertical layers) and to maintain a diverse vertical, horizontal and age structure of forest stands.

Classic selective thinning in suitable stand conditions is replaced by situational thinning, which is a significantly less risky way of caring for growing stands. With the increase in the frequency and severity of injuries, the risk of damage to stands also increases and thus the devaluation of the high investment in tending measures (ZGS, 2021).

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