LIFE SySTEMiC

Technical manual

A summary of scientific protocols

LIFE SySTEMiC

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LIFE SySTEMiC – Close to nature forest sustainable management practices under climate change

B. Implementation actions

Action B4: Replicability and transferability of project tools and results

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The purpose of the Life Systemic technical manual is to share protocols that were used in the LIFE SySTEMiC project for research activities on demonstration plots. This document contains a summary of scientific protocols for: forest structure and health, deadwood and microhabitats, sampling trees and their genetic analyses, sampling soil biodiversity and their analyses, browsing and planting experiment, structure of GenBioSilvi model and protocol for cost calculation. Detailed protocols are available on LIFE SySTEMiC web page: https://www.lifesystemic.eu/

It is desirable that innovative and reliable tools, in synergy with specific technical manual and specific guidelines in the sustainable forest management (SFM) field, are shared & replicated at the national & EU level in order to make them unique tools to deal more effectively and efficiently with the Governance of forest management, in line with the EU Forestry Policy.

The purpose of this action is to transfer and replicate the results and products of LIFE SySTEMiC to the stakeholders and end users at national & EU level.

Publication of technical manual, demonstration activities & creation of specific networking with other EU countries and projects will enable the transferability and replicability of the project results. These activities will guarantee the transferability and replicability of the project outputs at national level during the project and facilitate its continuation after its end. Projects experts' networking with other EU projects will also permit to transfer the methods and tools developed to other EU countries. Further value will be developed by a deep debate and exchange with other projects to better manage the protection and restoration of the EU forest and their sustainable management.

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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 LIFE SySTEMiC Project (Close-to-nature Forest Sustainable Management under Climate Changes) is to use the “modeling tool" based on genetic diversity to determine best silviculture practices in order 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 resistance and resilience of the forest system.

Based on these premises, the main project objectives are to:

- Investigate the relationships between forest management and genetic diversity for eight forest tree species in three European countries (Croatia, Italy, Slovenia) in order to identify the silvicultural systems that maintain high levels of genetic diversity.
- Develop 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.
- Spread 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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The LIFE SySTEMiC project tested the multidisciplinary approach ("forest structure-landscape genomics") in several protected and managed forests in different European forest types in three countries (Croatia, Italy, Slovenia) from the Alpine to the Mediterranean region. Standardized protocols were established to allow replicability and transferability of the approach and results to other European countries.

The purpose of the LIFE SySTEMiC technical manual is to share protocols that were used in the Life SySTEMiC project for research and demonstration activities carried out on demonstration plots.

This document contains a summary of scientific protocols for:

- demonstration area selected
- forest structure and health,
- deadwood and tree microhabitats,
- forest cutting
- sampling trees and their genetic analyses,
- sampling soil biodiversity and their analyses,
- browsing and planting experiment,
- structure of GenBioSilvi model and
- protocol for cost calculation.

Detailed protocols are available on Life Systemic web page: https://www.lifesystemic.eu/

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 fulfill 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 demonstration 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 main measures to adapt forest management to climate change are focusing on adaptation of tree composition in 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).

Table 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, entrance allowed only for research after obtaining a permit by the regional forest and nature conservation authority, IUCN conservation category I.
Low intensity management	Irregular shelterwood	Regeneration in openings and gaps (regeneration cores), where 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 acquires a new form with a multidimensional structure (adapted after Čater M., Diaci J. 2020); can be considered as IUCN conservation category IV or VI.
	Individual tree selection system	Trees of all dimensions are presented on a small surface area. 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. In the selection stands three main stand positions can be defined: suppressed, waiting individuals in the bottom layer, runners in the middle layer and winners (carriers) in canopy layer (Čater M., Diaci J. 2020); can be considered as 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 species being 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 understorey until the understorey no longer requires 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. Conversion can be done gradually or by clear-cutting followed by planting.
	Outside forest plantations	Planting of tree seedlings outside the forest area.

2. AREA SELECTION

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The activity was part of the action A1.2 Preparatory screening and implementation adjustments. This sub-action plays a key role in setting the background context against which set the stage for the implementation actions. It was devoted to screening of existing Dynamic Genetic Conservation Units (DCU) from the EUFGIS (European information system, www.eufgis.org), forest reserves & different categories of managed forests in participating countries for a correct implementation of other actions. Area selection was done for the selected 8 forest tree species in 3 countries. The selection of demonstration sites considered different European Forest Types (EFTs), managed at different intensities or unmanaged (forest reserves) in order to estimate the influence of forest management on forest structure and genetic spatial structure, as well as on soil biodiversity, and to enable the preparation of the GenBioSilvi model.

When choosing demonstration areas, we consider they could be set within the existing DCU (within the EUFGIS information system), networks of forest reserves (at the national level), and in different categories of managed forests (e.g., NATURA 2000 sites, approved seed objects, forests under different silvicultural systems) and silvicultural systems.

2.1 What to consider when choosing sites for demonstration plots

When choosing the area that will be included as a Life SySTEMiC demonstration site, different characteristics were taken into consideration:

- characteristics of the area (altitude, ground rock material, soil type, vegetation cover – phytocoenological association - potential natural tree species composition),
- actual composition of tree species – main tree species and other tree species present,
- growing stock and dead wood stock,
- age and origin of the stand, history of the stand,
- forest structure, presence of natural regeneration (saplings/cores),
- forest management / silvicultural regime,
- other important data (such as NFI plot, forest (genetic) monitoring plot, Natura 2000 site or else).

The area selected for the demonstration site should be representative of the whole stand, it means that it should have characteristics of the surrounding stand. When possible, natural regeneration should be present.

An overview of European forest types per country was performed, as well as an overview of EUFGIS DCUs within the country and an overview of forest reserves and other kinds of protected sites, such as Natura 2000, also considering selection of tree species and the

number of plots per species, providing any possible long-term monitoring and statistical analyses within and among other plots (species).

For better understanding we add here the overview of EUFGIS units in Europe (figure 1) and the location of demonstration plots in the LIFE SySTEMiC project (figure 2).

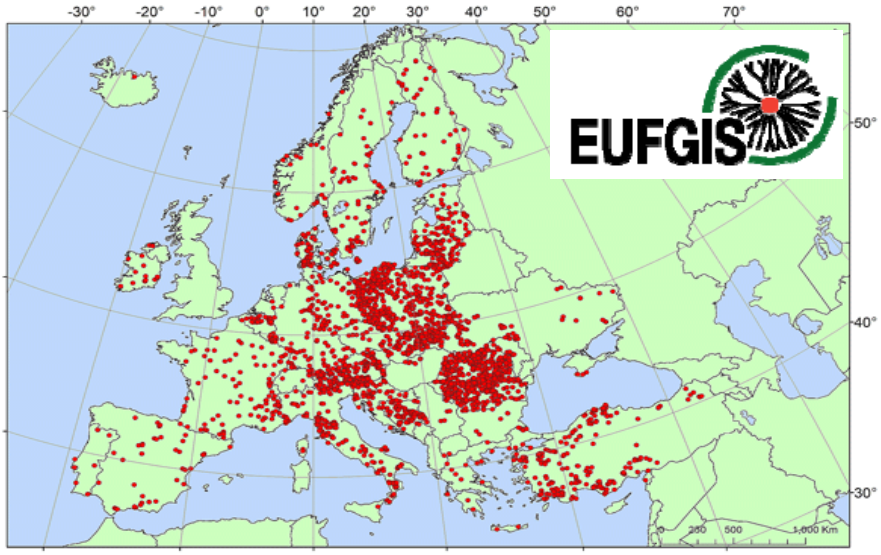

Figure 1: The EUFGIS dynamic genetic conservation units as from October 2020 (EUFGIS.org)

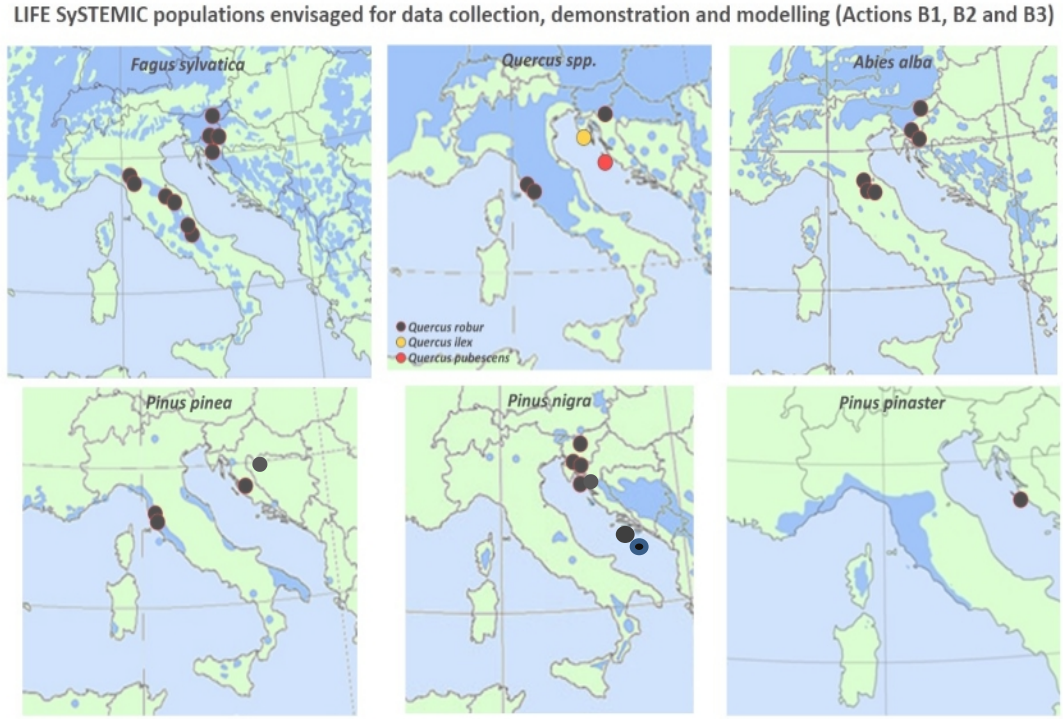

Figure 2: The overview of primary selection of demonstration plots per species and per country, presented within the EUFORGEN species distribution area maps (EUFORGEN.org)

In order to better represent unmanaged as well as disturbed sites, an additional plot in Croatia, for *Pinus pinea* on Mljet - plot n. 31 (national park) was selected. While the site in Krakovski gozd (Slovenia) was divided in two parts, one in an unmanaged forest reserve (plot 28B) and the other one in a low-intensity managed forest (plot 28A).

Table 2: The overview of Life SySTEMiC demonstration plots, presented by name of the plot, country, tree species, European forest type, stand structure and silvicultural system used.

Num	Site name	Country	Species	EFT *	Structure	Silvicultural system
1	Pian degli Ontani	Italy	*F. sylvatica*	7.3	Even-aged	Uniform shelterwood
2	Baldo's forest	Italy	*F. sylvatica*	7.3	Uneven-aged	Individual tree selection system
3	Pian dei Ciliegi	Italy	*F. sylvatica*	7.3	Even-aged	Uniform shelterwood
4	Caselle 1	Italy	*F. sylvatica*	7.3	Even-aged	Uniform shelterwood
5	Caselle 2	Italy	*F. sylvatica*	7.3	Even-aged	Uniform shelterwood
6	Faltelli	Italy	*A. alba*	10.6	Even-aged	Uniform shelterwood
7	Tre Termini	Italy	*A. alba*	7.3	Unven-aged	Individual tree selection system
8	Terminaccio	Italy	*P. pinea*	10.1	Even-aged	Clear-cutting and planting
9	Fossacci	Italy	*P. pinea*	10.1	Even-aged	Clear-cutting and planting
10	Culatta	Italy	*Q. robur*	5.1	Uneven-aged/ Unmanaged	Unmanaged
11	Fonte Novello	Italy	*F. sylvatica*	7.3	Uneven-aged/Old-growth	Unmanaged
12	Venacquaro	Italy	*F. sylvatica*	7.3	Even-aged	Uniform shelterwood
13	Nova Gradiška	Croatia	*Q. robur*	5.1	Even-aged	Uniform shelterwood
14	Ogulin	Croatia	*F. sylvatica*	7.2	Even-aged	Uniform shelterwood
15	Zadar	Croatia	*P. pinea*	10.1	Even-aged	Clear-cutting and planting
16	Gorski kotar, Skrad	Croatia	*A. alba*	3.2	Uneven-aged	Individual tree selection system
17	Klana	Croatia	*P. nigra*	3.3	Even-aged	Uniform shelterwood

Num	Site name	Country	Species	EFT *	Structure	Silvicultural system
18	Brač	Croatia	*P. nigra*	10.2	Even-aged	Irregular shelterwood
19	Pelješac	Croatia	*P. pinaster*	10.1	Even-aged	Irregular shelterwood
20	Pula	Croatia	*Q. ilex*	9.1	Even-aged	Uniform shelterwood
21	Črni kal	Slovenia	*Q. pubescens*	8.1	Even-aged	Irregular shelterwood
22	Mlake	Slovenia	*P. nigra*	14.1	Even-aged	Uniform shelterwood
23	Osankarica	Slovenia	*F. sylvatica*	7.2	Even-aged	Irregular shelterwood
24	Pri Studencu	Slovenia	*F. sylvatica*	6.6	Even-aged	Irregular shelterwood
25	Rajhenavski Rog	Slovenia	*F. sylvatica*	7.4	Uneven-aged/Old-growth	Unmanaged
26	Smolarjevo	Slovenia	*A. alba*	3.2	Uneven-aged	Individual tree selection system
27	Leskova dolina	Slovenia	*A. alba*	7.4	Even-aged	Irregular shelterwood
28A	Krakovo (Managed)	Slovenia	*Q. robur*	5.1	Even-aged	Uniform shelterwood
28B	Krakovo (Reserve)	Slovenia	*Q. robur*	5.1	Uneven-aged/ Unmanaged	Unmanaged
29	Gorski kotar, Vrbovsko	Croatia	*F. sylvatica*	7.2	Uneven-aged	Individual tree selection system
30	La Verna	Italy	*A. alba*	10.6	Uneven-aged/Old-growth	Unmanaged
31	Mljet	Croatia	*P. pinea*	10.1	Even-aged	Uniform shelterwood

* EFT = European Forest Type: 3.2 Subalpine and mountainous spruce and mountainous mixed spruce-silver fir forest; 3.3 Alpine Scots pine and Black pine forest; 5.1 Pedunculate oak-hornbeam forest; 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; 8.1 Downy oak forest; 9.1 Mediterranean evergreen oak 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

Information on demonstration sites is available at the Life SySTEMiC web page: https://www.lifesystemic.eu/demonstration-sites/

3. PROTOCOLS FOR FOREST STRUCTURE AND HEALTH

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Forest structure field manual provides detailed instructions for establishing a demonstration plot. It includes protocols for surveying live trees, identifying species, measuring breast height diameter, and positioning trees, measuring total tree height, height to base of live crown, and crown projection, defoliation, estimating volume and aboveground biomass at tree level, assessing data quality and providing data, and references. It includes the following field forms: Positioning of plot corners; Description of plot by visual assessment and Measurement of living tree are attached to the document.

First, a permanent plot with a representative structure of forest management has to be chosen. The plot has a rectangular shape and each side is 50 m long (area of 2500 m^2). At least 30 living trees (with diameter at breast height > 2.5 cm) of considered tree species have to be present on the plot. If there are less than 30 living trees, then one more plot has to be established in the demonstration site, until the minimum number of 30 trees is reached (Figure 3).

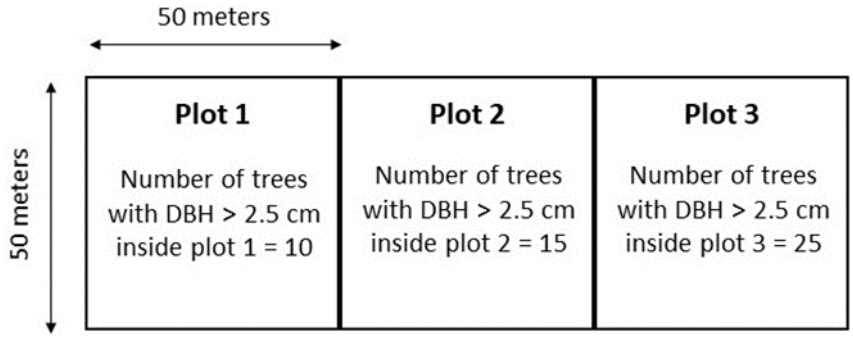

Figure 3: Plots layout in case there is less than 30 trees on first plot

Once the plot is established, the positioning of plot corners is performed, followed by description of the plot by visual assessment. In particular, the first information concerns the general data about the plot (European forest type classification, forest management and silvicultural system, silvicultural cuts carried out in the last 10 years, percentage of crown cover by vertical layers, and tree regeneration).

Then, data concerning the measurement of each living tree is reported: tree species, diameter at breast height (dbh), total tree height, height-to-base of live crown, crown projection and tree location. Each tree is marked and labeled with a unique code number (tree ID). Moreover, tree health is assessed by visual assessment of crown defoliation periodically or every year. Defoliation is divided into five classes (FAO, 2014) relative to a reference tree with full foliage (Table 3).

Table 3: Health status – defoliation classes

Degree of defoliation	Defoliation class	Percentage defoliation
None	0	0-10%
Slight	1	10-25%
Moderate	2	25-60%
Severe	3	60-100%
Dead tree	4	100%

For each living tree the volume and above ground biomass are calculated using National Forest Inventories double-entry volume tables.

4. PROTOCOLS FOR DEADWOOD AND TREE MICROHABITATS

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4.1. Protocol for deadwood measurements

The following dead wood components (ForestBIOTA, 2005; Travaglini *et al.*, 2007; Seidling *et al.*, 2014) are measured in the permanent plot:

- **Standing dead trees (including snags):** for standing dead trees the DBH and the total height are measured; for each snag (snag is standing dead tree with the stem broken at height ≥ 1.3 m) the diameter at the base of the stem, the diameter at the top of the stem, and the total height are measured. Snags with height < 1.3 m are classified as stumps.

- **Downed dead trees:** for each downed dead tree, the DBH and the total length are measured.
- **Other lying dead wood pieces:** for each lying dead wood piece the total length, the diameter at the base and the diameter at the top of the piece are measured.
- **Stumps (with a height < 1.3 m):** for each stump the diameter at the top, the diameter at the base and the total height are measured.

Tree species are determined for every deadwood component, also geographic coordinates are recorded, volume is calculated, and dead wood decay class is assessed (Figure 4) as reported by Hunter (1990).

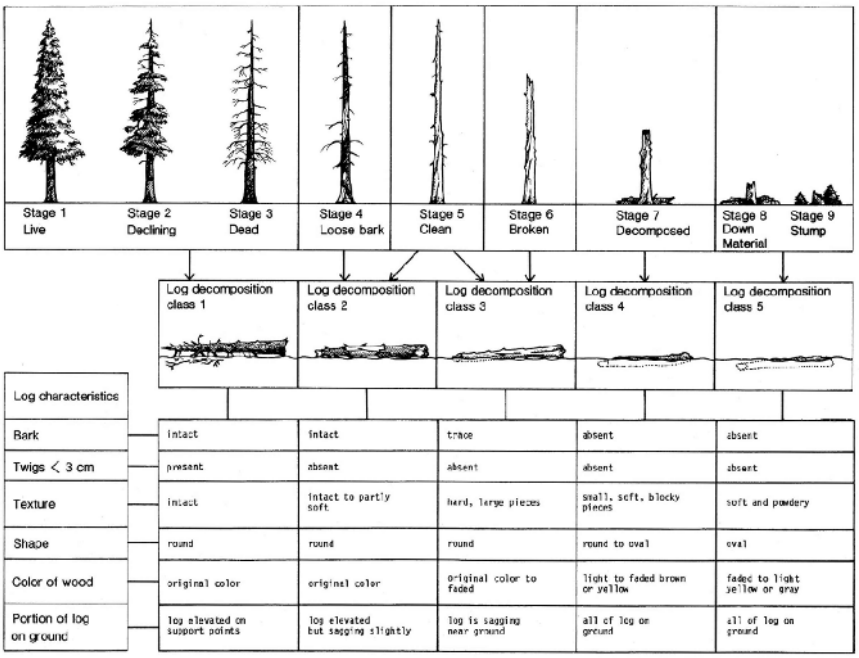


Figure 4: Deadwood decay classification (5 classes) system (Hunter, 1990)

4.2. Protocol for tree microhabitats inventory

The microhabitat inventory is performed using the protocol developed by Kraus *et al.* (2016). The classification of tree microhabitats consists of 8 forms, divided into several groups, distinguished by a code. Finally, each group is divided into types, for a total of 64 different tree microhabitats. Every type of tree microhabitat is identified, counted and reported in the Field Form.

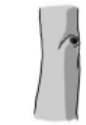
Illustrations	Code	Type	Description	Saproxylic microhabitats
CV3		Branch holes		Cavities
	CV31 CV32	ø ≥ 5 cm ø ≥ 10 cm	Rot-holes originating from branch breakage at trunk when fungal decay of wood is progressing faster than occlusion of wound.	
				
	CV33	Hollow branch, ø ≥ 10 cm	Hollow more or less horizontal branch developing from breakage. Provides tubular shelter from surrounding microclimate.	
CV4		Dendrotelms and water-filled holes		
	CV41 CV42	ø ≥ 3 cm / trunk base ø ≥ 15 cm / trunk base	The entrance diameter is the same as the chamber´s interior. Cup-shaped concavities that, due to their form, retain precipitation for a certain period of time (until it dries out).	
	CV43 CV44	ø ≥ 5cm / crown ø ≥ 15 cm / crown	The entrance diameter is the same as the chamber´s interior. Cup-shaped concavities that, due to their form, retain precipitation for a certain period of time (until it dries out).	

Figure 5: Example of tree microhabitats description from Catalogue of tree microhabitats (Kraus *et al.*, 2016)

5. FOREST CUTTING

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Forest cutting was carried out in Italy in 6 demonstration sites: site n. 2, site n. 3, site n. 6, site n. 7, site n. 8 and site n. 9.

Site n. 2 was an uneven-aged stand dominated by *F. sylvatica*, which was managed for timber production by a private owner using the individual tree selective thinning. This system was applied by the owner in 2021 for demonstration purposes. The number of trees per hectare before and after the cut was 2164 trees and 2072 trees, respectively. The growing stock volume per hectare before and after the cut was 363 m^3/ha and 300 m^3/ha, respectively.

Site n. 3 was an even-aged stand dominated by *F. sylvatica*. In 2021, the individual tree selective thinning was applied in this site to create gaps in the forest cover with the aim of favoring natural regeneration and the transition from even-aged to uneven-aged structure. The number of trees per hectare before and after the cut was 456 trees and 432 trees, respectively. The growing stock volume per hectare before and after the cut was 341 m^3/ha and 296 m^3/ha, respectively.

Site n. 6 was a young and very dense even-aged stand dominated by *A. alba*. In 2021 a thinning from below was carried out to reduce tree density and improve the stability of the stand. The number of trees per hectare before and after the cut was 1360 trees and 912 trees, respectively. The growing stock volume per hectare before and after the cut was 439 m^3/ha and 405 m^3/ha, respectively.

Site n. 7 was a mixed stand with *F. sylvatica, A. alba* and *Q. cerris*. In 2021 the individual tree selective thinning was applied to favor the growth of the natural regeneration already present in the stand. The number of trees per hectare before and after the cut was 1132 trees and 948 trees, respectively. The growing stock volume per hectare before and after the cutting was 501 m^3/ha and 426 m^3/ha, respectively.

Sites n. 8 and 9 were even-aged stands dominated by *P. pinea*. In the past, these forests were managed by clear-cutting followed by planting. In 2022, the clear cutting was carried out in site 8. Instead, in site 9 two experimental cuts were carried out as an alternative to clear-cutting. In particular, the uniform shelterwood system was carried out in the plot 9B and the group selection system was carried out in the plot 9C. In the plot 9B, the seed cut was

applied to favor both pine regeneration already present in the stand and new renewal growth; in this plot, the number of trees per hectare before and after the cut was 82 trees and 56 trees, respectively; the growing stock volume per hectare before and after the cutting was 366 m^3/ha and 265 m^3/ha, respectively. In the plot 9C, the group selection system was carried out also to favor the transition from even-aged to uneven-aged structure; in this plot the number of trees per hectare before and after the cut was 64 trees and 54 trees, respectively; the growing stock volume per hectare before and after the cut was 316 m^3/ha and 260 m^3/ha, respectively.

6. PROTOCOL FOR SAMPLING TREES AND THEIR GENETIC ANALYSES

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6.1 Genetic sampling strategy and DNA extraction protocol

LIFE SySTEMiC was developed to assess the impact of forest management on genetic variability to maintain and potentially increase it. Therefore, we need to evaluate which forest management practices maintain and potentially increase forest genetic structure through genetic recombination among adult individuals.
Published data indicate that old-growth beech forests developing for many decades in the absence of direct anthropogenic disturbances, present a complex genetic spatial structure which is non-random and where each subpopulation represents a family cluster (Paffetti et al 2012). Similar data has been found in beech forests under "single tree selection system", indicating that a forest can be managed without compromising its genetic structure and therefore maintains its "internal adaptation ability" in changing environments (Vettori et al 2024). Considering that it is generally accepted that genotypes tend to present a non-random distribution within natural populations (Epperson, 2000), we have simulated samples of different sizes following the stratified random sampling approach, in order to identify which is the sample size that provide results comparable with those obtained by Paffetti et al (2012) and possibly identify which was the best sample size to be adopted for landscape genetic analyses.
Censusing forest structure and collecting plant material can take a long time, and subsequent genetic analysis of all trees is time-consuming and costly. Following the data of Vettori *et al.* (2024) and Nijensohn *et al.* (2005), we can use stratified random sampling in SySTEMiC forest stands to conduct genetic landscape analyses and assess genetic structure as a function of forest management. This is also a strategy for landscape genetic studies that attempts to link processes that typically occur at different spatial and temporal

scales (e.g., changes in land use versus changes in genetic variation) (Lowe *et al.* 2004; Bankenhol and Fortin, 2016). If necessary, this sampling method can be used to reduce sample size without disturbing the structural pattern of the forest, which need not be altered by reducing the number of trees. The sampling strategy described in the protocol allows for a reduction in genetic cost without significantly affecting the final genetic data, allowing more stands to be analyzed for each species The protocol describes the stratified random sampling strategy for landscape analyses:

1. Unmanaged old-growth beech forest
2. Beech stand managed by uniform shelterwood system

with simulation of removal of different proportions of the original stock to reduce sample size. Following the simulation, genetic variability is estimated for each scenario.
The results obtained with the stratified random sampling show that the reduction in the number of trees to 30% of the total **for old growth forest** or **for managed forest areas** is representative of the census.

The protocol also includes a **sampling protocol**, a **DNA extraction protocol**, and **a DNA shipping protocol**. Sampling of tree material for DNA extraction must be completed prior to the start of the growing season, from trees selected according to the protocol described above. The sample from each tree must be placed in a Falcon tube (50 ml) or plastic bag with silica gel to preserve the material during sampling and for shipment. It is imperative that each falcon tube or plastic bag is labeled with the code ID corresponding to the tree measured in the forest structure.

6.2 Microsatellite analyses

Nuclear microsatellite markers (nSSR) were analysed in the LIFE SySTEMiC project as a measure of neutral genetic variation and structure of the studied populations. 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 and can confound the identification of true adaptive loci if not corrected for (Excoffier & Ray 2008; Excoffier *et al.* 2009; Sillanpää 2011). Microsatellite analyses can be divided in 5 major steps: (1) amplification of target nSSR markers using fluorophore-labeled primers in multiplexed PCR reactions; (2) dilution and denaturation of PCR amplicons; (3) fragment analysis - high-resolution capillary electrophoresis separation and detection of the amplified DNA fragments; (4) detected allele size calling; (5) data analysis - calculation of genetic variation parameters, determination of neutral genetic structure, etc. Guichoux *et al.* (2011) provide an excellent step-by-step overview of the microsatellite analysis.

The entire microsatellite analysis protocol, including the list of markers used for each species and PCR protocols, is available on the LIFE SySTEMiC web page: https://www.lifesystemic.eu/.

6.3 Adaptive genetic analyses

The purpose is to analyze the presence of a possible correlation between the allele frequencies of the studied populations and the climatic indicators under consideration.
We used a methodology based on the candidate gene approach to identify SNPs in individuals within the project's LIFE SySTEMiC sites of interest. The candidate gene approach involves three distinct phases: (1) a priori identification of candidate genes (genes relevant to abiotic stress response), (2) specific primer design and sequencing using Next Generation Sequencing (NGS) technology for Targeted Re-sequencing, and (3) SNP detection/calling. The workflow, split into the three phases, is summarized graphically below (Figure 6). This approach is less costly with respect to the NGS of whole genome and permits to focalize in those genes which have been demonstrated to be involved in the metabolic pathways important for response to climatic changes (Garosi *et al.* 2022).

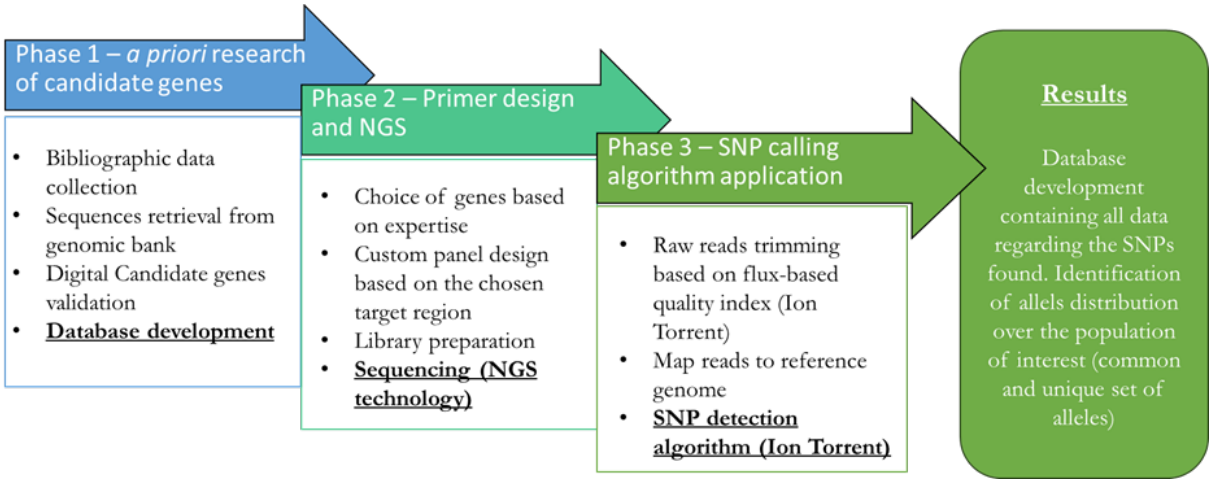

Figure 6: Candidate genes approach workflow followed for the analysis.

The approach allowed us to focus on specific target genomic regions that were chosen a priori for their relevance to abiotic stress response. The set of SNPs obtained for each population was analyzed to identify SNPs sets that might be relevant for adaptation to ongoing climate changes. This approach enables us to better understand the potential adaptability of the studied populations to changing environmental conditions.
The Genetic analysis strategy and data are available in the articles Garosi et al 2024a and Garosi et al 2024b (submitted) and as deliverables on the LIFE SySTEMiC web page: https://www.lifesystemic.eu/.

7. PROTOCOL FOR SAMPLING SOIL BIODIVERSITY AND THEIR ANALYSES

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7.1 Soil sampling for analyzing mycorrhiza and soil microbiome on Fagus sylvatica Life Systemic plots

The LIFE SySTEMiC project employs traditional and advanced techniques to identify ectomycorrhizal fungi and analyze soil microbiota, providing insights into forest ecosystem functions. Sampling procedures in the project are designed to capture the seasonal and spatial variability within forests. Samples are collected from multiple points around selected trees, ensuring a comprehensive representation of the local ecosystem. The timing of sampling, following the first autumn rains, is critical to capture the peak of microbial activity.

7.2 Detailed description of the sampling procedure

In each 0.25 ha sampling plot, five adult trees were selected, adjusting the number based on the expected diversity of the site and considering species accumulation curves. It was ensured that these trees were at least 10 m (one stand height) apart from trees of other ectomycorrhizal tree species. The coordinates of trees were recorded for accurate data mapping. Sampling for ectomycorrhizae and general microbiome was done concurrently, accompanied by the sampling of soil for physicochemical properties.

Soil samples were collected one meter away from the trunk of each selected adult tree. Around each of the five trees, four points in S, N, E and W directions were selected. At each of these four points, one subsample for ectomycorrhizae, one subsample for general microbiome and one subsample for soil analyses were collected, maintaining a distance of 10 cm between each subsample. Four subsamples from the S, N, E and W sampling points were merged into one composite sample per tree. Soil samples were collected by using the soil probe (2 cm in diameter), up to a depth of 30 cm, ensuring to remove larger pieces of decomposing organic matter while retaining the humus layer which is rich in fine roots and ectomycorrhizal fungi. After sampling around one tree and before moving to the next, the soil probe was cleaned with water and ethanol (70%) to prevent cross-contamination. There is no need for cleaning with ethanol between the sampling around a single tree.

7.3 Transport and storage of samples

Samples for identifying the dominant ectomycorrhizae were transported using ice packs, as they must be kept cooled to approx. + 4°C, but not frozen. In the lab, they should be kept refrigerated in closed plastic bags and processed as soon as possible. If samples for soil microbiome are expected to reach the laboratory within the same day and will subsequently be stored at -20°C, they can be transported using ice packs. Otherwise, the use of dry ice is recommended. Samples for soil analyses were kept at room temperature during the transport and later left to dry in the air on trays.

7.4 Morphoanatomical characterization and quantification of ectomycorrhizae

Roots from each sample for ectomycorrhizae are washed under tap water on a sieve with mesh opening 2 mm to obtain the fragments of roots. Ectomycorrhizal root tips are divided into morphotypes and quantified by counting under a stereomicroscope. Root fragments were randomly picked until a minimum of 250 vital ectomycorrhizal root tips were counted.

For each morphotype a preparation of the mantle was observed under the microscope to observe the taxonomically important anatomical structures (Agerer *et al.* 1987-2012). A representative mycorrhizal system of each morphotype was preserved in a freezer (-20°C) for a subsequent DNA extraction.

7.5 Molecular identification of ectomycorrhizae

Frozen ectomycorrhizal root tips were freeze-dried, homogenized and the DNA extraction was performed, using a commercial kit according to the manufacturer's instructions. The entire ITS region of ribosomal DNA in the genome of higher fungi was amplified by the polymerase chain reaction (PCR) using the fungal-specific primers ITS1f and ITS4r (White *et al.* 1990; Gardes and Bruns 1993, Sulzbacher *et al.* 2016). The amplified DNA fragments were separated using the agarose gel electrophoresis, purified and sequenced (Sanger). The obtained chromatograms were analyzed with suitable software and the obtained contigs identified by using BLASTN algorithm in the NCBI and the UNITE database.

Identities obtained by molecular methods were verified by comparing morpho anatomical features with descriptions of features for individual genera available in the literature (Agerer *et al.* 1987-2012).

7.6 Processing of samples for soil microbiome

Samples were freeze-dried and then homogenized. DNA was extracted using a standard commercial kit. To analyze the microbial communities present, the DNA was subjected to PCR amplification: the 16S rRNA V3 and V4 regions for bacterial communities, the ITS2 region for fungal communities, and the SSU V1-V4 regions for archaeal communities. The resulting PCR products are then indexed and purified before being sent for Illumina MiSeq sequencing.

Post-sequencing, the data underwent filtering and the sequences were clustered into operational taxonomic units (OTUs) using a 97% sequence similarity threshold (e.g., using Usearch, as implemented in SEED2 pipeline, Vetrovsky *et al.* 2018). Taxonomic identification of the representative sequences was performed by "blastn" algorithm against the UNITE database for fungi (Abarenkov *et al.* 2020) and the Silva database for bacteria and archaea (Quast *et al.* 2013), facilitating a comprehensive analysis of the entire microbial community.

7.7 Soil analyses

Soil analyses should provide at least information on soil pH , organic C and total N. Additionally, mineral C, exchangeable Na, K, Ca, Mg, Al, Fe, Mn and H+, cation exchange capacity, base cations and acid cations, saturation, extractable P, K, Ca and Mg can be analyzed. Analyses were performed on five composite samples per location according to the standardized procedures listed here:

8. PROTOCOLS FOR BROWSING AND PLANTING EXPERIMENTS

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8.1. Protocol for ungulate browsing experiment

To investigate the impact of wildlife browsing on genetic diversity, it is first necessary to locate sites in the chosen area with an acceptable density of natural regeneration (approx. 3200 saplings/ha). Two experimental plots should be set up in each site - one fenced and one unfenced, which will allow us to compare the genetic diversity of saplings subjected to

wildlife browsing to saplings not subjected to wildlife browsing. At least four pairs of plots representing four replications are required (Figure 7). Each plot has a size of 12.5 x 12.5 m.

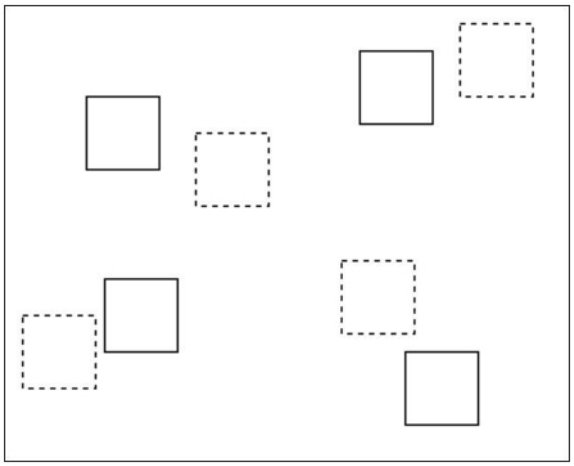

Figure 7: Scheme of experimental plots in pairs: one is fenced and the other is not.

The following plot characteristics are visually assessed once the plots have been established and before we begin measuring: rockiness, slope, inclination (cardinal direction: N, NE, E, SE, S, SW, W, NW), dominant tree layer per species, overtopped tree layer per species, young tree layer (to 2m height) per species and shrub layer.

Field measurements include sapling counting, sapling height measurement and browsing status assignment (Table 4).

Table 4: The status of wildlife browsing damage

Code	Status
0	No damage
1	Terminal shoot damaged
2	Lateral shoot damaged
3	Terminal and lateral shoots damaged

For genetic analysis, 50 sapling samples per plot should be collected. The samples should be collected evenly over the plot, to cover as much area as possible. Measurements and sampling should be performed at least twice with minimum tree-year gap.

8.2. Protocol for planting experiment: oak powdery mildew control protocol

Oak powdery mildew (*Erysiphe alphitoides*) is one of the most serious diseases of oak (*Quercus robur, Q. petraea*) in Europe. Its proliferation is particularly damaging on young trees, where it limits growth and causes significant mortality. The experiment's goal is to determine how planting density and various AQ-10 biopesticide concentrations affect powdery mildew infection in seedlings. The effects of planting density will be evaluated using two different planting densities, roughly 1100 and 4400 seedlings per hectare, and the effects of biological control will be evaluated using two different concentrations of AQ-10, half the concentration and the concentration recommended in the instructions and the control.

The experiment should be conducted in 4 blocks, with each block containing all combinations of the specified fixed factors (2 x 3 = 6 treatments). A split-plot experimental design should be utilized, as seen in Figure 8.

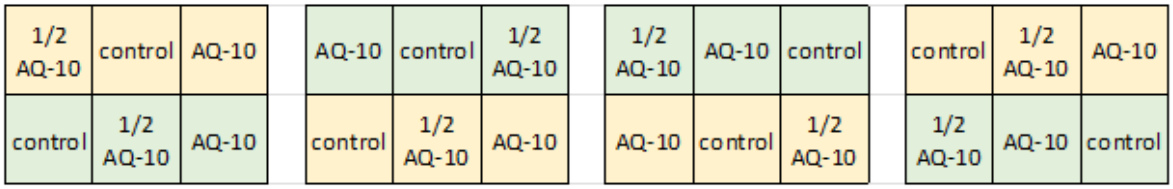

Figure 8: Split-plot experimental design scheme with randomly assigned treatments; planting density: 1100 seedlings / ha - yellow, 4400 seedlings / ha - green

Each plot is 22.5 by 36 meters in size. There should be clear markings indicating the denser (1.5 x 1.5 m) and sparser (3 x 3 m) planting densities. After that, 960 seedlings – 240 for each block - are planted. The planting should be carried out manually by digging planting pits. When planting, the roots should be evenly distributed over the pit. To assure optimal root contact with the soil, the soil surrounding the seedling needs to be gently compacted with the foot. Every seedling that is planted needs a stake to provide support.

Seedlings are sprayed with AQ-10 in the spring when the first signs of infection appear. The spray slurry should be prepared according to the biopesticide's instructions; in our case 70g/ha for full concentration and 35g/ha for half concentration. Spraying is carried out twice with a 7–10 day interval.

Measurements of all seedlings' heights from the ground to the top of the tallest shoot should be made either in the spring before the growing season begins or in the fall after the growing season has ended. The measurements of height are repeated annually.

In addition to height measurements, a visual inspection of seedling infestation is carried out once a year in mid-September, and each seedling is rated on a 5-point scale:

0 - healthy leaves without any signs

1 - small infestation, with powdery mildew covering up to 25% of the leaf surface

2 - moderate infestation, with powdery mildew covering 26–50% of the leaf surface

3 - strong attack; powdery mildew covers 51–75% of the leaf surface

4 - extremely severe attack; powdery mildew covers more than 76% of the leaf surface.

9. GENBIOSILVI MODEL STRUCTURE

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2. D.R.E.A.M., Italy
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We have developed the GenBioSilvi model as a tool for Sustainable Forest management. The loss of biodiversity at local, regional, national, and global levels is a reality (Fussi *et al.*, 2016). The two main forces that cause of loss of biodiversity are anthropic activities and climate changes. The utilization of indicators is pivotal in monitoring and describing biodiversity across all levels. The indicator is defined as a measure or metric based on verifiable data that conveys information about more than itself. Typically, indicators are periodically measured to reflect changes relative to predefined objectives. Criteria and verificators are required to define indicators. Criteria can be defined as standards against which assessments are made, reflecting specific purposes or goals. Verifiers, on the other hand, entail parametric data and serve as direct measures of the indicators.

To implement the predictive model GenBioSilvi for sustainable forest management, for genetic diversity, forest structure, deadwood, soil diversity, and microhabitat indicators are calculated. Each indicator was calculated for each species included in the project, namely

Fagus sylvatica L., *Abies alba* Mill., *Quercus robur* L., *Quercus ilex* L., *Quercus pubescens* Willd., *Pinus nigra* J.F. Arnold, *Pinus pinea* L. and *Pinus pinaster* Aiton. To implement ecosystem biodiversity indicators, six criteria were considered: (i) Stand Structure, (ii) Deadwood, (iii) Species Diversity, (iv) Genetic Diversity, (v) Soil Microbiome Diversity, (vi) Microhabitat.

Considering the results obtained from the analysis of all biodiversity indicators, we developed a model that describe the status of genetic resources within the site. This model was developed to help forest users for checking the 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 be observed.

In this regard, we have developed a model that involves the observation of some key indicators that describe the status of the analysed stand.

Table 5. 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 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 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.

10. PROTOCOL FOR COSTS CALCULATION

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The cost calculation protocol was developed to facilitate the transferability process. The costs for site identification, sampling, genetic analysis, cutting and browsing experiments were recorded separately in each country and are included in the Exploitation plan part 2 (B4 deliverable). Here we only provide a brief overview.

All actions and sub-actions carried out at the sites involve traveling and subsistence. The costs for the **identification of demonstration sites** and the costs for the **determination of the forest structure** only consist of travel and subsistence costs, all other categories also include labor and material costs. To calculate the costs for the individual actions and sub-actions, we indicate the cost categories and the estimated labor and material costs. In the Exploitation plan part 2, the costs for LIFE SySTEMiC are listed by action.

Taking soil samples from 5 trees per plot, for 3 types (ECTO, PEDO, MICRO) includes 15 combined samples per plot. For each tree we sampled 4 directions N, S, W, E, which means 60 subsamples. Dry ice is needed, but the consumption can be lower if it is dried and reused.

The material for **tree sampling** includes silica gel and bags for leaves. Consumption depends on the number of trees sampled. The cost of thinning consists of fencing, surveys, sampling, data analysis and material costs. The **fencing** and survey also require traveling and subsistence costs. Fencing for **browsing**: The time required for a 12.5 m x 12.5 m plot varies depending on the terrain and land. The approximate times are: Fencing: 1 - 2 hours (2 people); Fencing: 4 - 7 hours (3 people) or more if the plot still needs to be selected. Browsing survey: Time required for a plot of 12.5 m x 12.5 m: approx. 6 hours (2 persons). Taking seedling samples: twice per project, for one sampling: 16 hours (2 persons). **Data analysis for browsing:** Time needed per analysis: approximately 5 hours. **Planting** Costs of planting consist of travel, subsistence costs and planting material costs.

The **costs of genetic analysis** consist of costs for DNA extraction, molecular analyses, data analysis, microsatellite. They are evaluated per sample or in hours needed to analyze one sample.

Costs of soil biodiversity analysis consist of:

- **Soil microbiome** that includes average time for laboratory work (0.5 hours/sample): approximately 0.2 hours/sample for DNA extraction, 0.3 hours/sample for PCR reactions, including indexing for sequencing, and subsequent cleaning of PCR product**; DNA extraction per sample** and **molecular analyses. Data analyses** takes approximately 0.3 hours /sample. **Soil microbiome analysis** (sequencing): the standard is 30 bioinformatics hours per 2-100 samples (16S + ITS2), so 0.3 hours /sample

- Ectomycorrhizae that includes cleaning and morphotyping- for each morphotype morphological-anatomical analysis and molecular analyses were performed; **Anatomical and Morphological analysis:** DNA extraction (includes sample preparation, tissue disruption and DNA extraction with Qiagen DNeasy Plant Pro kit), Molecular analyses (includes PCR amplification, agarose gel electrophoresis, PCR product purification, Sanger sequencing (sense and anti-sense) and 10% technical overage), data analyses from sequencing (includes sequence quality control (Phred), contig assembly, BLASTN search in UNITE and GenBank nucleotide sequence databases, preparation of tables); **Integrative analysis:** matching morpho-anatomical analyses with molecular data: Hours/sample 24h per plot (i.e. 5 samples).

Cutting was only carried out in Italy; we indicate the costs incurred in different locations. Demonstration site name:

- **Fossacci (Italy):** External service 7524,80 € / ha
- **Pian dei Ciliegi (Italy):** Pian dei Ciliegi cutting of crop choice, intervention surface 2500 square meters, intervention cost €/ha 7.278,81 (of which € 2.099.33 for supplies and € 5.179.48 for labor). hours used/ha: 264.
- **Faltelli (Italy**): Faltelli thinning intervention area 2500 square meters, intervention cost €/ha 7.516,65 (of which € 1.933,73 for supplies and € 5.582.93 for labor); hours/ha: 360
- **Tre Termini (Italy**): Tre Termini crop choice cutting, intervention surface 2500 square metres, intervention cost €/ha 7.516,65 (of which € 1.933,73 for supplies and € 5.582.93 for labor); hours/ha: 360
- **Baldo's stand (Italy):** External service 5.737,71 € / ha (without vat).

11. REFERENCES

Abarenkov K., Zirk A., Piirmann T., Pöhönen R., Ivanov F., Nilsson R.H., Kõljalg U. (2020): UNITE QIIME release for fungi. UNITE Community. 10.15156/BIO/786385

Agerer R. (1987–2012) Colour atlas of ectomycorrhizae. Einhorn, Schwäbisch-Gmünd

Bajc M., Aravanopoulos F, Westergren M., Fussi B., Kavaliauskas D., Alizoti P., Kiourtsis F., Kraigher H. 2020. *Manual for forest genetic monitoring*. 1st ed. Ljubljana: Slovenian Forestry Institute, Silva Slovenica Publishing Centre. 326 pp. Studia Forestalia Slovenica, 167. ISBN 978-961-6993-56-2. ISSN 0353-6025. http://dx.doi.org/10.20315/SFS.167, http://dirros.openscience.si/IzpisGradiva.php?id=13902, DiRROS - Digitalni repozitorij raziskovalnih organizacij Slovenije, DOI: 10.20315/SFS.167. [COBISS.SI-ID 42816515]

Bankenhol N., Fortin M.J. 2016. Basics of study design: sampling landscape heterogeneity and genetic variation for landscape genetic studies. In: Bankenhol N, Cushman S, Storfer A, Waits L (eds). Landscape genetics: concepts, methods, applications. Wiley, Chichester, pp 58–76. doi:10.1002/9781118525258.ch05.

Čater M., Diaci J. 2020. Forest management - silvicultural systems. In: Forests and forestry in Slovenia. Ed: Čater M, Železnik P., Slovenian Forestry Institute, The Silva Slovenica Publishing Centre: 120 p.

Excoffier L., Ray N. (2008) Surfing during population expansions promotes genetic revolutions and structuration. Trends in Ecology & Evolution, 23, 347–351.

Excoffier L., Hofer T., Foll M. (2009) Detecting loci under selection in a hierarchically structured population. Heredity, 103, 285–298.

Epperson B.K. (2000). Spatial genetic structure and non-equilibrium demographics within plant populations. Plant Species Biology 15, 269-279. doi: 10.1046/j.1442-1984.2000.00046.x

FAO (2014). Manual for visual assessment of forest crown condition. Pristina, 2014. http://www.fao.org/3/a-i4214e.pdf.

ForestBIOTA (2005). Stand structure assessments including deadwood within the EU/ICP Forests biodiversity test-phase (ForestBIOTA). Forest-BIOTA - a forest biodiversity monitoring project developed by 10 European countries, Thünen Institute ed, Braunschweig, Germany, pp. 17. [online] URL: http://www.forestbiota.org/docs/cccstruct1_revMay06.pdf.

Forest management by mimicking nature, How to conserve forests by using them. 2014. Slovenia Forest Service: 27 p.

Gardes M., Bruns T.D. (1993) ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113–118.

Garosi C, Ferrante R, Vettori C, Paffetti D. 2022. Meta Analysis as a tool to identify candidate genes involved in the Fagus sylvatica L. abiotic stress response. Forests 13, 159. doi:10.3390/f13020159

Garosi C., Paffetti D., Conti S., Vettori C. 2024a. Development of a primer set for *Fagus sylvatica* gene sequencing and evaluation of Torrent Suite Variant Caller analyses. (Submitted)

Garosi C., Vettori C., Arcidiaco L., Bajc M., Kraigher H., Westergren M., Dovč N., Travaglini D., Damjanić R., Sever K., Breznikar A., Gregoric A., Lanšćak M., Ivankovic M., Bogunović S., Paffetti D. (2024b). Genome-wide SNP association analysis reveals genomic signature of local adaptation in European beech (*Fagus sylvatica* L.). (In preparation)

Guichoux E., Lagache L., Wagner S., Chaumeil P., Leger P., Lepais O., Lepoittevin C., Malausa T., Revardel E., Salin F., Petit R.J. (2011) Current trends in microsatellite genotyping. Mol Ecol Resources, 11, 591-611. doi: 10.1111/j.1755-0998.2011.03014.x

Hunter ML. (1990). Wildlife, forests, and forestry: principles of managing forests for biological diversity. Englewood Cliffs, N.J., Prentice Hall. 370 pp.

Kraigher H., Bajc M., Božič G., Brus R., Jarni K., Westergren M. 2019. Forests, forestry and the Slovenian forest genetic resources programme. V: Šijačić-Nikolić M. (ed.), Milovanović J.(ed.), Nonić M. (ed.). *Forests of Southeast Europe under a changing climate*. 1st ed. Cham: Springer International Publishing AG, 2019, cop. 2018. pp. 29-47. Advances in Global Change Research, 65. ISBN 978-3-319-95266-6. ISSN 1574-0919. https://doi.org/10.1007/978-3-319-95267-3_3, DOI: 10.1007/978-3-319-95267-3_3. [COBISS.SI-ID 5272230].

Kraus D., Bütler R., Krumm F., Lachat T., Larrieu L., Mergner U., Paillet Y., Rydkvist T., Schuck A., Winter S., 2016. Catalogue of tree microhabitats – reference field list Integrate + Technical Paper, European Forest Institute, Freiburg, Germany.

Lowe A.J., Harris S.A., Ashton P.A. 2004. Markers and sampling in ecological genetics, pp. 6–51. In Ecological genetics: design, analysis, and application. Blackwell Publishing, Oxford, United Kingdom.

Nijensohn S.E., Schaberg P. G., Hawley G. J., DeHayes D. H. 2005. Genetic subpopulation structuring and its implications in a mature eastern white pine stand. Can J For Res 35, 1041-1052.

Paffetti D., Travaglini D., Buonamici A., Nocentini, S., Vendramin G.G., Giannini R., Vettori C. 2012. The influence of forest management on beech (Fagus sylvatica L.) stand structure and genetic diversity. For Ecol Manag 284, 34 – 44. doi: 10.1016/j.foreco.2012.07.026

Poljanec A., Bončina A. 2020. Structure and composition of forest stands at regional and national levels in the last five decades. In: Forests and forestry in Slovenia. Ed: Čater M, Železnik P., Slovenian Forestry Institute, The Silva Slovenica Publishing Centre: 120 p.

Quast C., Pruesse E., Yilmaz P., Gerken J., Schweer T., Yarza P., Peplies J., Glöckner F.O. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41(Database issue):D590-6.

Rellstab C., Gugerli F., Eckert A.J., Hancock A.M., Holderegger R. (2015) A practical guide to environmental association analysis in landscape genomics. Mol Ecol, 24, 4348–4370. doi: 10.1111/mec.13322

Seidling W., Travaglini D., Meyer P., Waldner P., Fischer R., Granke O., Chirici G.,Corona P. (2014). Dead wood and stand structure - relationships for forest plots across Europe. iForest, 7: 269-281.

Sillanpää M.J. (2011) Overview of techniques to account for confounding due to population stratification and cryptic relatedness in genomic data association analyses. Heredity, 106, 511–519.

Sulzbacher M.A., Grebenc T., García M.Á., Silva B.D., Silveira A., Antoniolli Z.I., Marinho P., Münzenberger B., Telleria M.T., Baseia I.G., Martín M.P. (2016) Molecular and morphological analyses confirm *Rhizopogon verii* as a widely distributed ectomycorrhizal false truffle in Europe, and its presence in South America. Mycorrhiza 26(5):377-88.

Travaglini D., Barbati A., Chirici G., Lombardi F., Marchetti M., Corona P. (2007). ForestBIOTA data on deadwood monitoring in Europe. Plant Biosystems, 141: 222-230.

Vetrovský T., Baldrian P., Morais D. (2018) SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. Bioinformatics 34(13):2292-2294.

Vettori C., Travaglini D., Bottalico F., Nocentini S., Matteucci D., Paffetti D. (2024a). Does silviculture influence genetic diversity in beech stands? New results suggest hypothesis for Genetic Conservation Unit management.(In preparation)

Vettori C., Travaglini D., Nocentini S., Paffetti D. (2024b). Can spatially explicit forest structure be used as an indicator of genetic sampling? (In preparation).

White T., Bruns T., Lee S., Taylor J., Innis M., Gelfand D. *et al.* (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR - protocols and applications – a laboratory manual. Academic Press: pp. 315–322.

***Abies alba*, Leskova dolina - Photo R. Damjanić**
The demonstration site is located at the foot of Snežnik mountain. In the continuous forests of Snežnik, Dinaric forests of beech and fir are predominant. The demonstration plot lies within the forest seed object and the forest genetic reserve for silver fir and is also a Special Protection Area and Special Area of Conservation of the Natura 2000 Network “Javorniki - Snežnik”.

***Fagus sylvatica*, Osankarica - Photo K. Sever**
The demonstration site lies within the forest seed object and forest gene reserve for beech. It is also under the Special Protection Area of the Natura 2000 Network named “Pohorje”. The entire forest seed object covers 8 ha and lies at an altitude of 1240 m.a.s.l.

***Pinus nigra*, Klana - Photo M. Lanšćak**
The demonstration site is located in forest seed object and gene reserve of *Pinus nigra* L. It is even-aged forest with stock reserve higher than in regular forest. The ground rock is brown soil on limestone and dolomites. Total area of forest seed object is almost 11 ha on altitude of 600 m.a.s.l.

***Quercus pubescens*, Črni Kal - Photo K. Sever**
The demonstration area is a part of the Natura 2000 Network – Special Protection Area and Special Area of Conservation “Kras”. The area is distinctly karst, which is indicated by high rockiness (up to 35%) and diverse relief. The plot is located on a slope, the ground rock is limestone.

***Pinus pinea*, Fossacci - Photo D.Travaglini**
The demonstration site is located in the Migliarino, San Rossore, Massaciuccoli Regional Park (Italy). The study area falls within a Special Area of Conservation and a Special Protection Area of the Natura 2000 Network, both named “Selva Pisana”. The forest in the demonstration site is an uneven-aged Stone pine (*Pinus pinea* L.) stand with natural regeneration of pine.

***Pinus pinaster*, Peljesac - Photo D.Travaglini**
The demonstration site in past was a forest seed object which was deleted from register after big forest fire in 2015. Now it is monitored as after-fire site with good results of natural regeneration. Now it is monitored as after-fire site with good results of nature regeneration. It is also under the Special Protection Area of the Natura 2000 Network named “Srednjedalmatinski otoci i Pelješac (Central Dalmatian islands and Peljesac)”.

***Quercus robur*, Krakovo - Photo D.Travaglini**
Krakovo forest is a wetland flooded by the Krka River. One demonstration site is located in the Krakovo forest reserve, which is protected as a forest reserve and is also under the SAC and SPA of the Natura 2000 Network. 2nd demonstration site is located in sorounding managed forest.

***Quercus ilex*, Pula - Photo M. Lanšćak**
The demonstration site is a part of forest seed object and gene reserve of *Quercus ilex* L., also a part of Mediterranean evergren oak forest area on the south part of Istria Peninsula. It is also under the Special Protection Area of the Natura 2000 Network named “Luka Budava - Istra”. Ground rock is limestone and area of forest seed object is 15 ha.

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