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Deliverable D3.2

Genetic, phenotypic and environmental characterisation of two GCUs for the remaining species considered in WP3

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Workpackage leader: INIA

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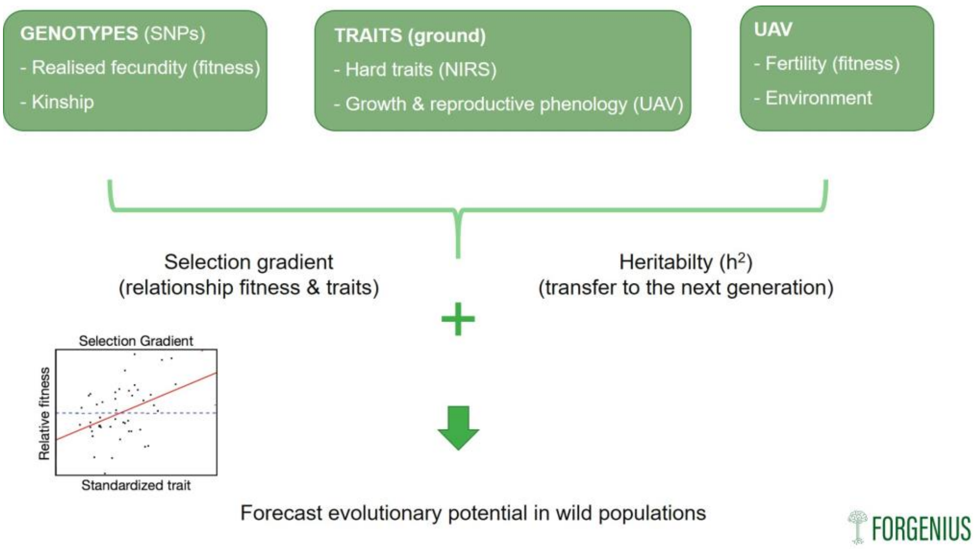
1 Summary

In the first three years of the project, WP3 has focused on producing three datasets (genetic, phenotypic and environmental data) for four forest tree species (*Populus nigra*, *Pinus pinaster, Fagus sylvatica* and *Pinus sylvestris*). The initial idea was to focus on only one species, but we changed our approach to take advantage of the fact that data are cumulatively produced. This strategy has paid off as we could get nearly complete datasets for the two focal species (*P. nigra* and *P. pinaster*) using a number of innovative methods, while datasets for the other two species will be completed by summer 2024 (delays are explained below). These datasets will contribute to the assessment of several population-level estimators (e.g. fitness, kinship, heritability) that will be incorporated into quantitative models aiming at predicting population adaptation in the wild (see the schematic representation of the work flow below), and thereby produce dynamic descriptors of Genetic Conservation Units (GCUs).



2 Introduction

For each of the four species, two GCUs with contrasted environmental settings have been characterized, and their characteristics are described in the Table 1 and Figure 1. Each GCU is composed of 500 adult trees (georeferenced at the individual level, see Figure 2) and 250 juvenile trees (georeferenced at the patch or individual level).

Table 1. Characteristics of the eight GCUs sampled in 2021-2023.

Species	EUGIS code	Country	Latitude	Longitude	# adults	# juveniles
P. nigra	AUT00284	Austria	48,18068-48,15283	16,49609-16,92818	500	250
P. nigra	ESP00395	Spain	41.584117	-0.762574	500	250
P. pinaster	FRA00051	France	44°57'48.6"N	1°09'53.9"W	500	250
P. pinaster	ITA00019	Italy	43.131365	11.248482	500	250
F. sylvatica	FRA00045	France	44º54'47.7966''N	5º14'6.8742''E	500	250
F. sylvatica	SVN00047	Slovenia	46.4317	13.6159	500	250
P. sylvestris	GBR00001	United Kingdom	57°36'17"N	5°18'43"W	500	250
P. sylvestris	FIN00001	Finland	61°51'40.0"N	29°22'54.1"E	500	250

Figure 1. Geographic distribution of GCUs across Europe, illustrating the locations where environmental data collection and analysis are being conducted

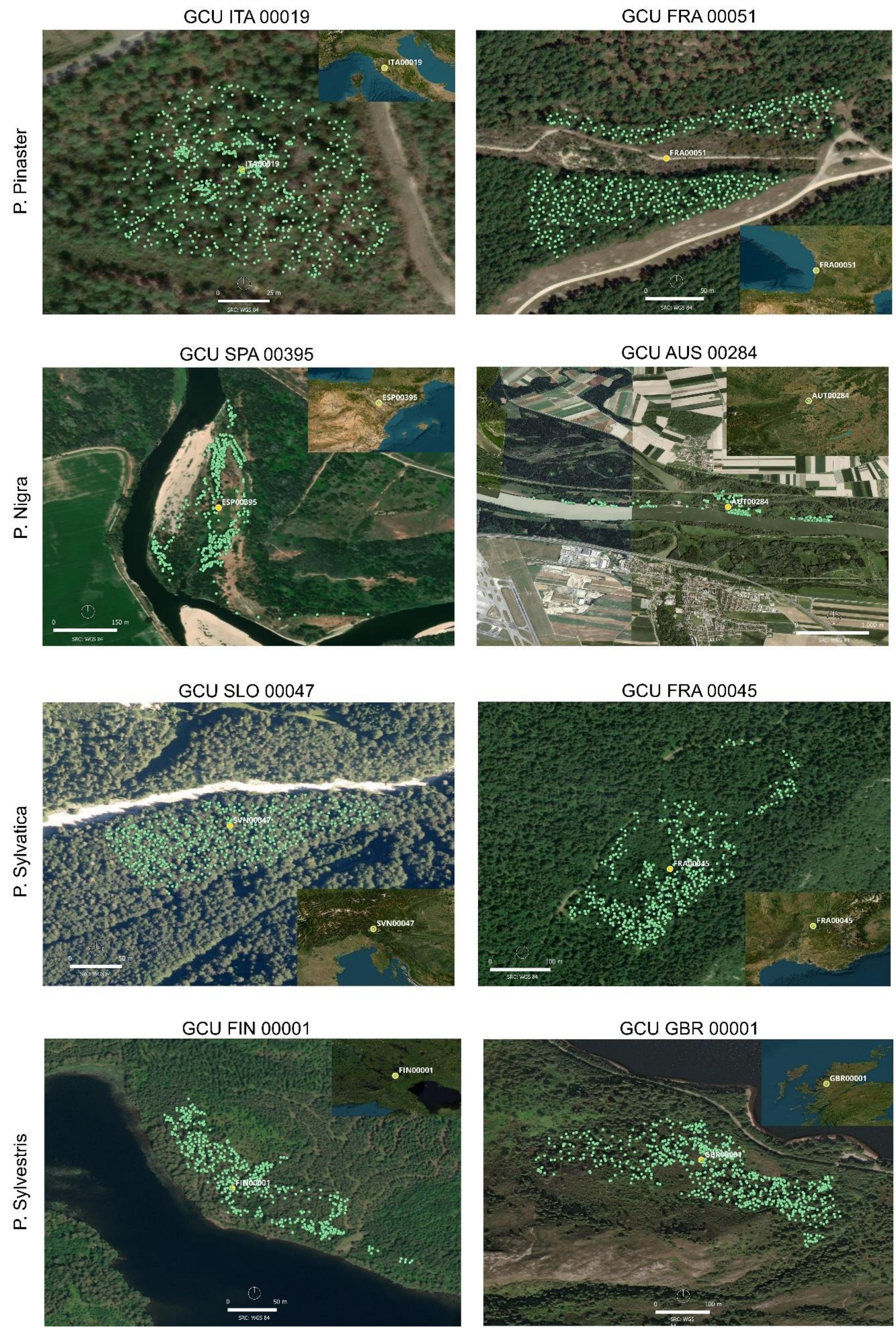


Figure 2: Adult tree distribution map for the eight GCUs sampled in between 2021-2023. Accurate positions were obtained by UMR using dGPS and tachymeters (see the “Phenotypic data” section below).

The 8 GCUs have been characterized by several datasets that are described afterwards: genetic datasets (for both adults and juveniles), phenotypic datasets measured from the ground at individual tree level (adults), and phenotypic and environmental datasets measured with unmanned aerial vehicle (adults).

3 Results

3.1 Genetic datasets

Leaf/needle tissue were collected by local partners for 2×500 adults and 2×250 juvenile trees per species. DNA was extracted by local partners then sent to CNR for handling and shipping to ThermoFisher Scientific and IGATech for genotyping. For three species (*P. nigra*, *P. pinaster* and *P. sylvestris*), the genotyping technology was the Axiom non-human microarray, using the SNP set 4TREE for the two first species (developed in the previous European project B4EST), while using the Pisy50K SNP-array for the latter species. Available genotypes and estimated delivery dates of the genotypes by the ThermoFisher Scientific Company are indicated in Table 2. For *F. sylvatica* the production of genotyping data was delayed because the company in charge (IGATech) did not provide the array design yet (all DNA samples are available). The genotyping of *P. sylvestris* is in progress and the data are expected to be delivered by the company within few months.

Table 2. Genotyping advances for adult and juvenile trees for the eight GCUs.

Species	Country	500 adult genotypes	250 juvenile genotypes
P. nigra	Austria	Available	Available
P. nigra	Spain	Available	Available
P. pinaster	France	Available	Available
P. pinaster	Italy	Available	Available
F. sylvatica	France	Available by May 2024	Available by May 2024
F. sylvatica	Slovenia	Available by May 2024	Available by May 2024
P. sylvestris	United Kingdom	Available by January 2024	Available by January 2024
P. sylvestris	Finland	Available by January 2024	Available by January 2024

⇨ Genomic datasets are held by CNR.

Extra genotyping was performed for the two GCUs of *Populus nigra* in order to determine unambiguously the sex of the trees: original sex determination was done based on flowering, a method that can introduce biases (i.e. males and females flowers are beige and should be assessed at the right period of the year to be able to distinguish them). Since this information is crucial to perform the analyses by sex, all 1,000 trees were genotyped following the protocol of Geraldes et al. 2015 (Molecular Ecology 24, 3243–3256).

⇨ Genetic datasets for sex determination are held by BFW and INIA-CSIC

3.2 Phenotypic datasets measured from the ground

For each of the four species, a set of traits were measured from the ground on adult trees by local teams along with the WP2 mobile team:

- ***Diameters at breast height of 2×500 trees***
  ⇨ Traits are held by local partners.

- ***Tree height of 2×500 trees***
  ⇨ Traits are held by local partners.

- ***Short increment cores*** were collected from 2×500 trees for *P. nigra*, *P. sylvestris* and *F. sylvatica*, while ***drill dust*** was collected for from 2×500 *P. pinaster* adult trees. From this material, Near InfraRed Spectroscopy (NIRS) measurements were performed (Table 3). Using a correlative approach linking the wood traits measured in WP2 across the full species range (150 trees) and the NIRS measured in WP3 (1,000 trees), we have been exploring whether we could infer wood traits for the 1,000 trees/species in WP3.
- ***Short cores of 10×15 trees*** were collected in WP2 and sent to INRAE in WP3 to collect NIRS spectra on solid and ground samples (Table 3).
- ***Branches of 10×15 trees*** were collected and provided by WP2 team for NIRS measurements (Table 3).
- ***Long increment cores of 2×50 trees***. From this material we measured X-ray microdensity profiles of the full core as well as of the outermost 5 cm. Using a correlative approach linking microdensity measured in WP2 across the full species range (150 trees) and NIRS measured in WP3 (1,000 trees), we have been exploring whether we can indirectly infer wood density for the 1,000 trees/species in WP3 (Table 3).

Table 3. Measurements of WP2 traits used for NIRS calibration

Species	Country	150 wood NIRS spectra for calibration	200 Wood microdensity for calibration	11 wood traits WP2 for calibration	150 branch NIRS spectra for calibration
P. nigra	Austria	Available	Available	Available	Available
P. nigra	Spain	Available	Available	Available	Available
P. pinaster	France	Available	Available	Available	Available
P. pinaster	Italy	Available	Available	Available	Available
F. sylvatica	France	Available by 01/2024	Available	Available	Available by 01/2024
F. sylvatica	Slovenia	Available by 01/2024	Available	Available	Available by 01/2024
P. sylvestris	United Kingdom	Available by 01/2024	Available	Available	Available by 01/2024
P. sylvestris	Finland	Available by 01/2024	Available	Available	Available by 01/2024

- **Functional traits measured by WP2 in 10 trees from 15 GCUs spanning the full distribution of the species**. We selected 11 of the most relevant traits to test for relationships between NIRS measurements and hard traits, and thereby derive hard traits for 2x500 trees. The list and characteristics of these traits are provided below (Table 4). Traits were measured using specific protocols for each trait (detailed in Deliverable 2.1 of

WP2), following Pérez-Harguindeguy et al (2013), Bartlett et al (2012), Burlett et al (2022), Beckett et al (2023).

Table 4. List and characteristics of the 11 traits selected from WP2.

Function	Traits	Ranking*	Definition
Survival during drought	*P50*	1	Water potential causing 50% of embolism.
Survival during drought	*Slope*	1	Slope parameters of the vulnerability to cavitation curves.
Growth and productivity	*wood density*	1	wood density.
Growth and productivity	*Ksmax*	1	Specific hydraulic conductivity (per unit sapwood area).
Survival during drought	*HV*	1	Huber value: sap wood area to leaf area ratio.
Survival during drought	*Capacitance*	1	Branch capacitance.
Survival during drought	*Capacity_branch*	1	Amount of water stored in the branch at saturation.
Survival during drought	*TLP*	2	Turgor loss point.
Survival during drought	*gmin*	2	Minimum or cuticular conductance.
Growth and productivity	*SLA*	2	specific leaf area.
Growth and productivity	*N*	2	Nitrogen content.

* We rank level 2 traits that are not measured on wood or traits less relevant.

NIR-traits model development and NIR traits prediction.

In order to characterize 2x500 adult trees for hard traits we used the following approach (Figure 3): 1) we measured hard traits on branch across a species distribution (10 trees in 15 GCUs = 150 trees) as well as NIRS on branch and on trunk for the same trees, 3) we developed a NIR model, linking NIRS with traits in the 150 trees, 4) we applied this model to the NIRS measurements of the 2 GCUs (500 trees in 2 GCUs = 1,000 trees) and used the calibration model to infer hard traits for 1,000 trees. This approach would allow to indirectly measure hard traits in many trees by sampling trunk samples and measuring only NIRS on them.

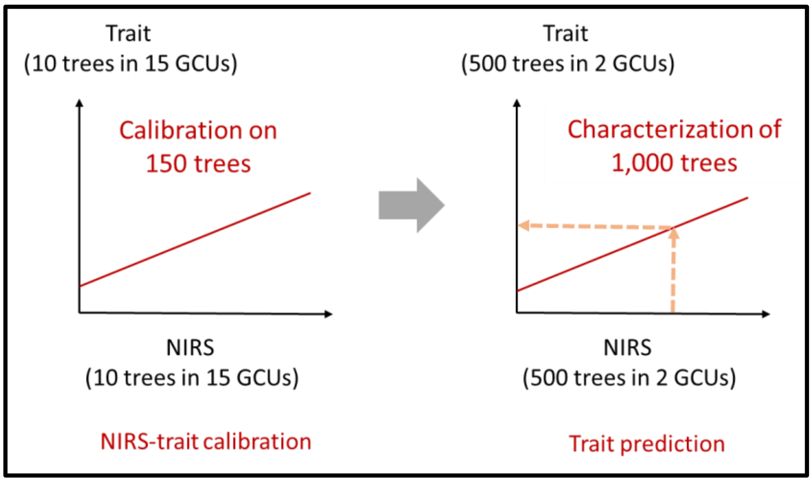


Figure 3: workflow to infer hard traits in the 2x500 trees. Left graph represents NIRS-trait calibration on 150 trees; Right graph represents trait prediction on 1,000 trees.

- *Scanning and study of drill dust samples (P. pinaster, CETEMAS)*

150 samples were scanned in the NIR and the corresponding spectra were matched to the hard traits values measured on the associated branch samples then grouped in a database. Despite several trials with different data analysis methods (transformations) applied to the NIR spectra, the calibration models obtained were not deemed sufficiently robust. In another trial, we also tested the models independently at provenance level, with no further success. Some models showed a promisingly high calibration coefficient of discrimination, but a low cross-validation coefficient of discrimination. In some cases, we unsuccessfully tested the deletion of a large number of samples.

- *Scanning and study of solid cores (P. pinaster, CETEMAS)*

As the results obtained with dust were inconclusive, we tested the calibration models with NIRS measured on solid samples: those that had been used to measure the density of the same trees with the ITRAX approach. We built the corresponding databases and retested the calibration models using different data analysis approaches (transformations), but the results were still inconclusive as they were not robust enough.

- *Scanning of drill dust samples milled (P. pinaster, CETEMAS)*

After the study of the drill dust samples, and taking into account the results of the FTIR from INRAE, the drill dust samples were milled, and scanned on two different ways, 1) using the “contact probe and small cup” method and 2) using the “rapid probe and vial” method. As in the previous cases, a database containing these spectra as well as the traits’ values measured on branch samples was built. Then different transformations of the spectra were tested, but the models obtained were once again considered not robust enough.

- *Scanning of branch milled samples (P. pinaster, CETEMAS)*

Given the results obtained with the drill dust samples and the solid trunk samples, and following a coordination meeting with INRAE staff, we decided to scan the branch samples, which are the same as those scanned by INRAE with the FTIR instrument.

- *Scanning of short cores and branches (INRAE Orleans – Phenobois platfom)*

All NIR spectra were acquired using an FT-NIR spectrometer (Frontier, Perkin Elmer) in the range 10000 to 4000 cm-1 (1000 - 2500 nm). Our strategy is based on the comparison of predictive models based on spectra collected on solid (short cores and branches) and ground samples. All analyses were performed using R Studio 4.3.1.

Our analytical method is based reducing the noise naturally present in the dataset. Then we carry on an exploratory analysis based on a principal component analysis. This step helps to check spectra clustering based on known factors (species, country and GCU). We then identify and remove outlier samples based on the mahalanobis distance.

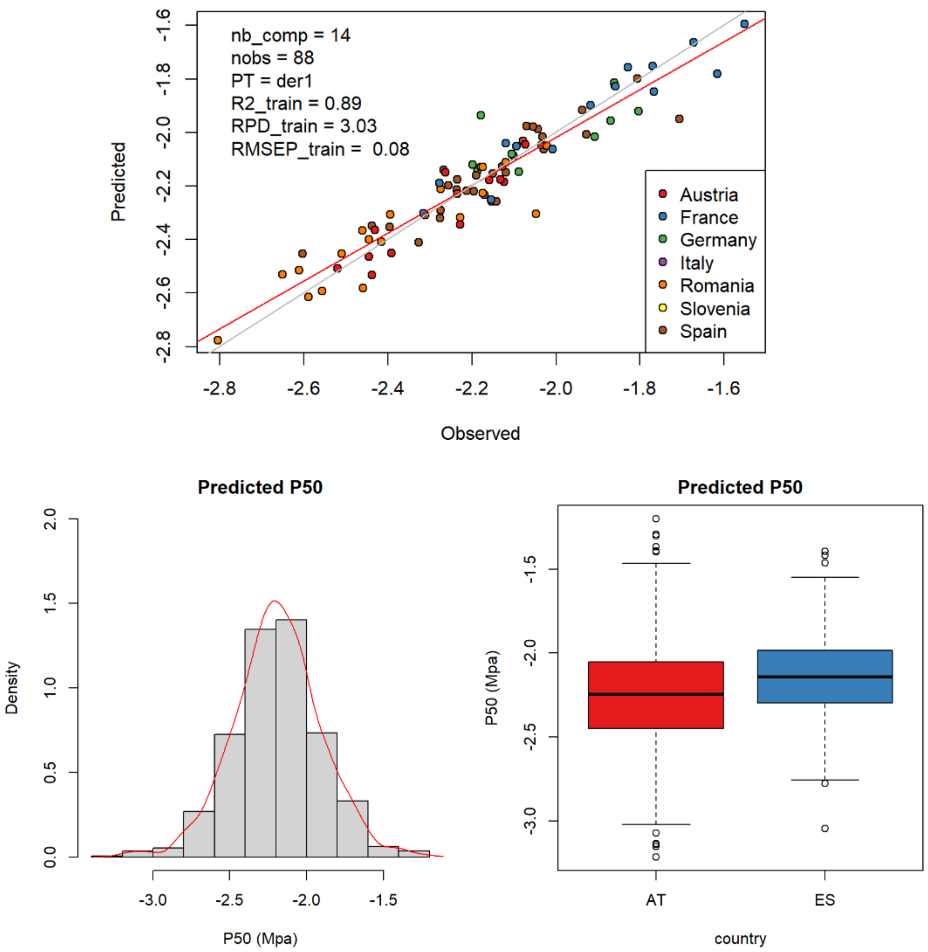
After cleaning the data set, we move to the model's training using the phenotypic traits measured and provided by WP2.

- *Populus nigra*

INRAE Orleans received 13 GCU samples (approximately 10 samples each) out of the 15 GCUS listed in the project. Missing GCUs are: ESP00395, FRA00073, FRA00074.

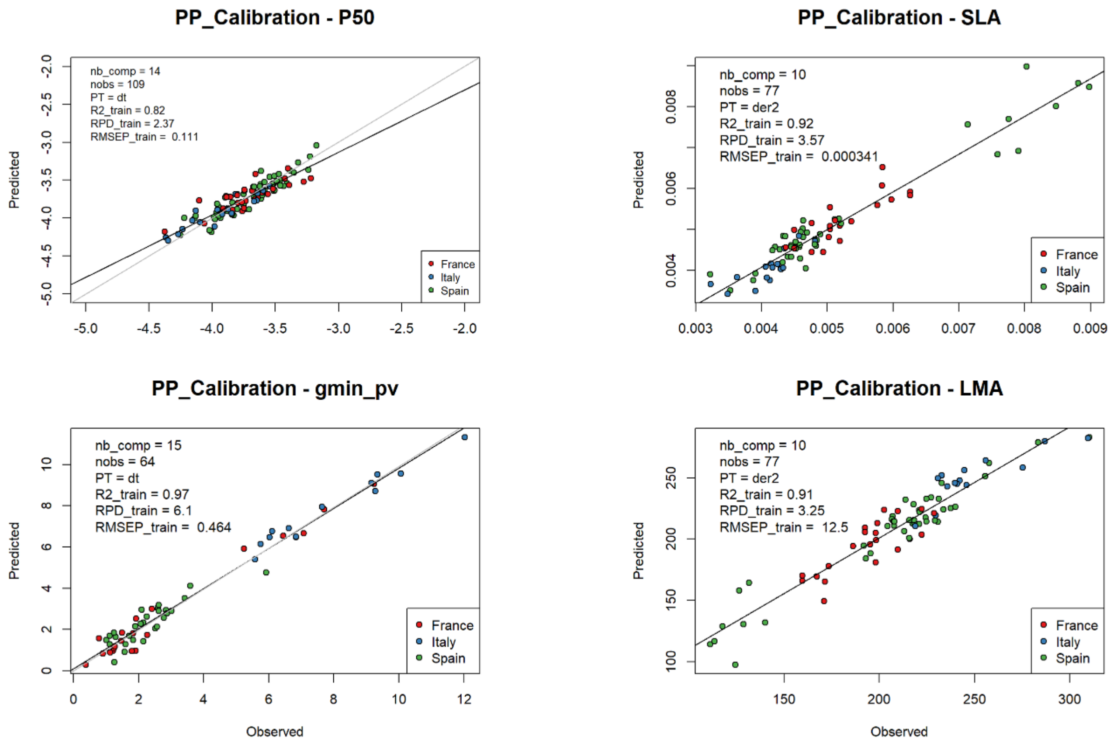
Given the reduced number of samples, we have opted for the use of a cross-validation method to select the models to be applied on all GCUs (AUT00284 and ESP00395).

We focused on the traits listed above but only P50 responded positively (based on ground short cores spectra) with a calibration R^2 of 0.89, an error of prediction RMSE of 0.08 (to be compared to the error of measurement in the lab). This model was applied of the 2x500 ground short cores spectra of Austrian and Spanish complete GCUs. These results have been made available to all interested parties.



- *Pinus pinaster*

Initially, we were in charge of collecting spectra on branches only (solid and ground). Models development resulted in the following results:



On the basis of these results, we confirm the predictive potential of some cavitation traits and highlight new predictable ones: SLA, LMA and Gmin.

Since CETEMAS has so far found no relationship for maritime pine (FRA00051 and ITA00063) using wood dust, analyses are underway to check whether this result is linked to biological or technical reasons. CETEMAS and INRAE Orléans have exchanged samples to test different protocols. INRAE Orléans is currently analyzing these samples to build new models and apply them to all GCUs.

- *Fagus sylvatica and Pinus sylvestris*

We started acquiring NIR spectra on both species, but had to interrupt the process in order to carry out the task described above for *Pinus pinaster* sawdust.

⇨ NIRS measurements, model development and predicted hard traits are held by CETEMAS and INRAE.

In conclusion, at the time of writing this deliverable, we have a minimum number of traits already measured on the ground samples for each of the four species (Table 5), plus other traits measured by UAV (Table 8).

Table 5. Summary of the traits measured in the eight GCUs

Species	**Country**	**500 diameters**	**500 heights**	**500 wood NIRS spectra**	**500 hard traits (WP2)**
P. nigra	Austria	Available	Available	Available	P50
P. nigra	Spain	Available	Available	Available	P50
P. pinaster	France	Available	Available	Available	SLA and Gmin will be predicted by the end of 2023
P. pinaster	Italy	Available	Available	Available	SLA and Gmin will be predicted by the end of 2023
F. sylvatica	France	Available	Available	Available	Available by april 2024
F. sylvatica	Slovenia	Available	Available	Available	Available by april 2024
P. sylvestris	UK	Available	Available	Available	Available by april 2024
P. sylvestris	Finland	Available	Available	Available	Available by april 2024

3.3 Phenotypic and environmental datasets from UAV

Effective monitoring of forest ecosystems with drones requires a meticulously planned strategy that integrates the principle of reproducibility at every stage of the process. This encompasses everything from the meticulous planning of flights and their precise execution to the careful processing of the captured images. The standardization of methods ensures that data sets collected in different locations or at different times are consistent and comparable, allowing for their integration and joint analysis.

Data Collection based on UAV, trigonometric and soil measurements (data level 0)

Drone flights (UAV) were conducted at the locations detailed in Figure 2 for data collection (data level 0) (see Table 6). To ensure geospatial precision, ground control point (GCP) surveys were conducted at each GCU, which were used for photogrammetric georeferencing. Field missions also included repeated measurements of soil moisture content (SWC) and soil temperature.

Meteorological challenges played a significant role in the planning of the missions, with extreme conditions forecasted by British meteorological agencies leading to the cancellation of the GBR0001 mission scheduled for October 2023. Even the tree measurement for FRA00045 was not completed due to difficult weather during the sampling week, so the remaining 10% is scheduled to be completed in early 2024. Additionally, due to the complexity of the sampling areas and phenological desynchronization between leaf and seeds development, it was necessary to repeat and perform multiple flights for data acquisition in ITA00019, FRA00051, ESP00395, and AUT00284.

Although no data was collected for GBR0001, we have a database provided by local collaborators that includes dense LiDAR clouds, trees measured with GNSS systems in RENIX format, and RGB images. These dense clouds are planned to be used to calculate morphological traits, although the precision and quality of the labeled trees remain unknown.

Data collection, including low-altitude UAV imagery for cone classification and soil parameters, as well as tree measurement, is scheduled to be completed in the summer of 2024 for the GCU of GBR0001.

Table 6. Data Level 0 collection overview for all GCUs, detailing the collection dates of tree measurements, environmental images, fertility images, and soil measurements. The visuals provide a snapshot of the diverse data gathered for forest GCUs assessment, with "Pending" indicating incomplete soil measurements and Fertility images data for GCU GBR00001.

DATA LEVEL 0 (Data Collection)

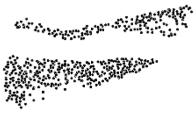
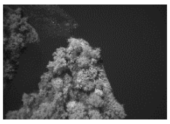
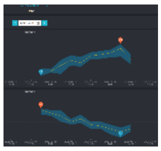
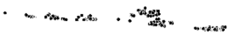
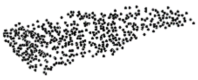
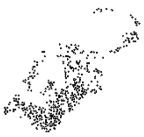
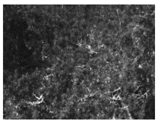
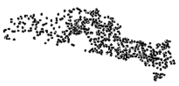
GCU	Date	Tree Measurements	Environmental Images	Fertility Images	Soil Measurements
ITA00019	2022-11-23				
FRA00051	2022-11-19				
ESP00395	2023-03-18 & 2023-09-07		 		
AUT00284	2023-03-30 & 2023-10-02			 	
SVN00047	2023-08-21				
FRA00045	2023-09-06				
FIN00001	2023-09-15		 	 	
GBR00001	25/05/2022 (by local partners)		 	Pending	Pending

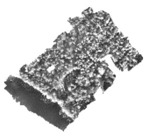
Image processing (data level 1)

Using high-resolution images captured from UAV platforms, we have generated dense point clouds to derive environmental variables and morphological traits, as well as orthoimages with resolutions ranging from 1 to 5 mm for seeds classification (see Table 7).

The photogrammetric processing of environmental and fertility images has been completed for ITA00019, FRA00051, ESP00395, AUT00284, and SVN00047. The environmental image processing for FRA00045 and FIN0001 has been completed. Additionally, the processing of LiDAR dense clouds derived from GBR0001 and the testing of the image quality of the RGB images from this same GCU are pending.

In the recent image processing phase for various GCUs, it is noteworthy that AUS00284 required significantly more computing time. While most GCUs completed photogrammetric processing in less than 24 hours, AUT00284 demanded 146 hours, reflecting the complexity and data volume collected for this specific location. This increase in processing time underscores the intensity of the work needed to transform UAV images into valuable information for the analysis of morphological traits and environmental variables.

Table 7. Data Level 1 image processing table for all GCUs. "Pending" indicates that processing for GCU GBR00001 is yet to be completed.

DATA LEVEL 1 (Image processing)					
GCU	Environmental Images			Fertility Images	
	Orthoimage	Dense Cloud (3D)	Time processing	Orthoimage	Time processing
ITA00019			3 h		22 h
FRA00051			2.5 h		9 h
ESP00395			16 h		6 h
AUT00284			146 h		5 h
SVN00047			59 h		2 h
FRA00045			35 h		1 h
FIN00001			4 h	 	2.5 h
GBR 00001	Pending	Pending		Pending	

Environmental variables and fertility processing based on UAV data (data level 2)

Morphological traits based in tree crown (ITC) and UAV dense clouds

The delineation of individual tree crowns (ITC) was estimated from dense 3D point clouds. ITC is crucial for recording cone productivity per tree. The delineated ITCs were automatically compared to the trees measured in the field using DGPS. ITCs not only record the position of the tree crown but also generate a list of morphological traits based on crown measurements projected onto a 2D canopy.

To generate surface models, the 3D point clouds series were classified into ground / non-ground points. Based on spline interpolation and iterative filtering, a Digital Terrain Model (DTM) was calculated from the ground points. After normalization of the values, a Canopy Height Model (CHM) was generated.

Environmental variables (Solar Energy, Morphometry and Hydrology)

Derived environmental parameters, such as those related to solar energy, hydrology, and morphometry, are calculated from generated digital surface models (DTM and Digital Surface Model - DSM) and a series of intermediate products. This calculation is performed using established GIS software tools such as SAGA, GRASS, and QGIS, along with several custom scripts in RStudio. The status of specific procedures and the input data used to calculate these variables are described in Table 8, located in the solar energy, hydrology, and morphometry sections of the table.

Soil parameters

To derive soil parameters dependent on topography, we employed the ordinary kriging with external drift (KED) method along with topographical data and a set of *in situ* measurements of soil water content (SWC) and soil temperature. The status of specific procedures and the input data used to calculate both variables are described in Table 8, located in the soil parameters sections of the table.

Seeds Classification

At the beginning of the project, we initiated the recording of cone productivity in the GCU of ITA00019 and FRA00051 using a machine learning (ML) approach implemented through the Weka segmentation tool. Although this approach proved to be efficient in detecting cone structures, we faced challenges related to the reproducibility of the method. In addition, we encountered limitations in the automated detection of seeds with morphological characteristics different from cones, such as green catkins, especially in the GCU of ESP00395 and AUT00284. This outcome underscores the need for additional adjustments in our methodology to adapt to a wider range of seed morphological characteristics.

For this reason, we incorporated an advanced method based on Meyer et al. 2018 - 2022 for the classification of tree seeds, using high-resolution (5 mm) UAV-derived RGB images to cover forest units. This big data process involves significant challenges in terms of computational time and memory consumption, which requires rigorous optimization of the model. Given the intense demand for computing resources, the outcome initially focused on a representative area of green and red catkins from the GCU ESP00395, with plans to run the complete model for the GCU of ESP00395 and a section of AUT00284 in December 2023, following additional adjustments to efficiently manage the data volume.

The workflow described by Goralogia et al. 2021, which is used to estimate catkin productivity from ground images, could certainly be refined to meet the specific challenges presented by the GCU of AUT00284. Capturing UAV images at low altitude over such an extensive area as 4 km, seeking homogeneous lighting conditions and within a short catkin development period, poses a considerable challenge. Logistical and meteorological constraints, such as the need for a week of flight under consistent weather conditions and without crosswinds, render this method impractical.

In response to these challenges, we opted for a terrestrial approach, capturing images from various angles. The selection of representative images and the estimation of catkin production follow the workflow of Goralogia et al. 2021, which involves categorizing the presence and abundance of catkins according to a defined scale. This method allows for a detailed assessment of catkin productivity directly from the ground, which may be more feasible under the given constraints.

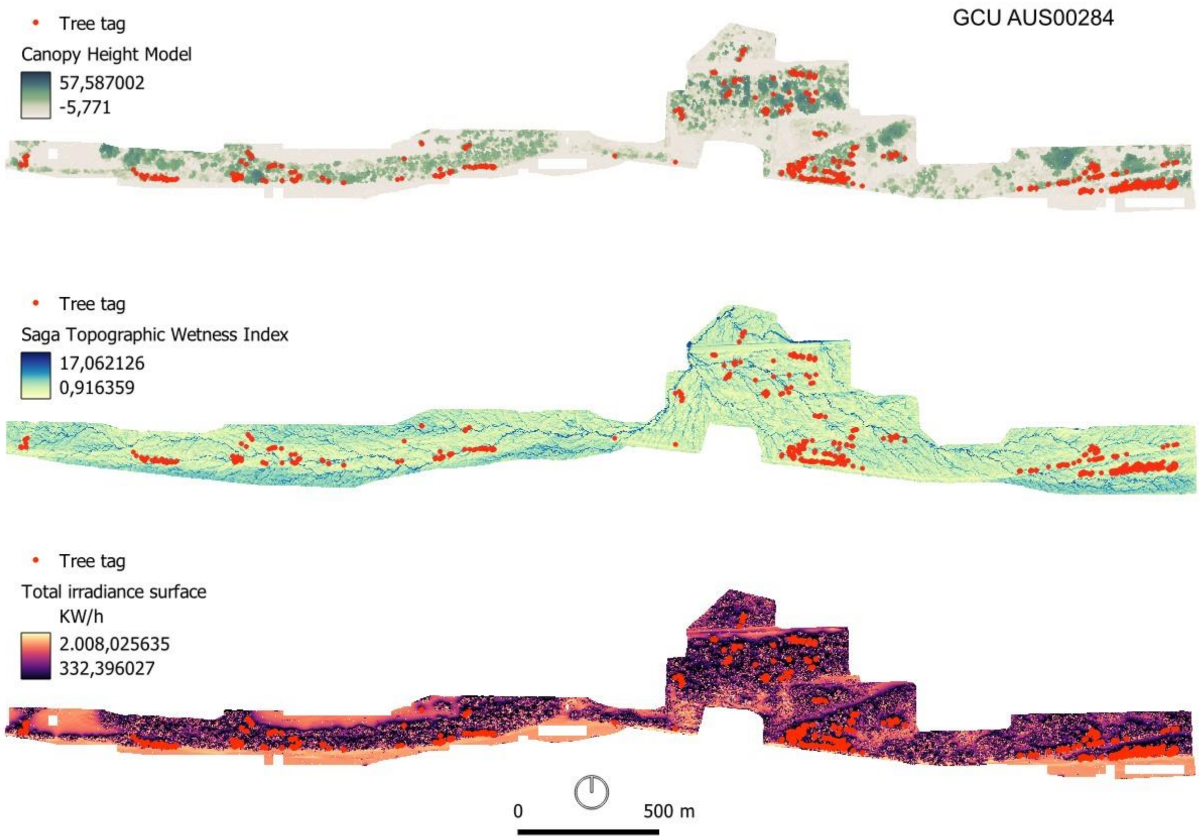
To validate this approach, comparing the automated classification of UAV images with the classification based on ground images will provide an assessment of the effectiveness and accuracy of both methods. This comparison will also help to understand the advantages and limitations of remote classification methods versus ground-based observation approaches, thus contributing to the continuous improvement of forest monitoring techniques.

Table 8. Overview of processing levels for processing (level 2) and deliverable environmental, morphological traits, and seed classification products across various GCUs. 'Available' indicates completed datasets, 'Pending' denotes datasets awaiting processing, and 'In testing' reflects ongoing testing. Variables marked with an asterisk (*) are computationally intensive, requiring over 200 hours of processing and significant memory resources, between 100 and 250 GiB of RAM and storage space.

Environmental, morphological traits and seeds classification processing (Data level 2) and deliverables									
Input	Outcomes	ITA00019	FRA00051	ESP00395	AUT00284*	SVN00047	FRA00045	FIN00001	GBR00001
3D Clouds	**Morphological traits**	Available	Available	Available	Available	Pending	Pending	Pending	Pending
	Environmental variables								
	Solar Energy								
DSM	Sky View Factor	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DSM	Direct Irradiance*	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DSM	Diffuse Irradiance*	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DSM	Global Irradiance*	Available	Available	Available	Available	Pending	Pending	Pending	Pending
	Morphometry	Available	Available	Available	Available	Pending	Pending	Pending	Pending
3D Clouds	DEM*	Available	Available	Available	Available	Pending	Pending	Pending	Pending
3D Clouds	Canopy Height*	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DTM	DTM slope	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DSM	DSM slope	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DTM	DTM aspect	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DSM	DSM aspect	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DTM	TRI	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DTM	TPI	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DTM	Curvature	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DTM	Convexity	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DTM	Terrain Class.	Available	Available	Available	Available	Pending	Pending	Pending	Pending
	Hydrology	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DTM	STWI*	Available	Available	Available	Available	Pending	Pending	Pending	Pending
DTM	SWI	Available	Available	Available	Available	Pending	Pending	Pending	Pending
	Soil Parameters	Available	Available	Available	Available	Pending	Pending	Pending	Pending
Soil Measure.	SWC	Available	Available	Available	Available	Pending	Pending	Pending	Pending
	Soil Temp.	Available	Available	Available	Available	Pending	Pending	Pending	Pending

orthoimage	**Seeds Classification***	Available	Available	In testing	Available	Pending	Pending	Pending	Pending

Here we present a series of maps (Figure 4) that exemplify the analysis of environmental variables processed for the GCU AUT00284 and ESP00395. For instance, the Canopy Height Model provides a visual representation of the height variation among the trees in both GCUs, using red dots to mark the sampled trees. Concurrently, the SAGA Topographic Wetness Index delivers valuable information on areas prone to moisture accumulation, with more intense colors suggesting zones with a higher potential for water retention, a factor that directly impacts forest growth and health. Additionally, the Total Irradiance Surface highlights the differences in solar energy reception, with darker areas indicating higher irradiation, which is essential for processes such as photosynthesis and thus for ecosystem productivity.



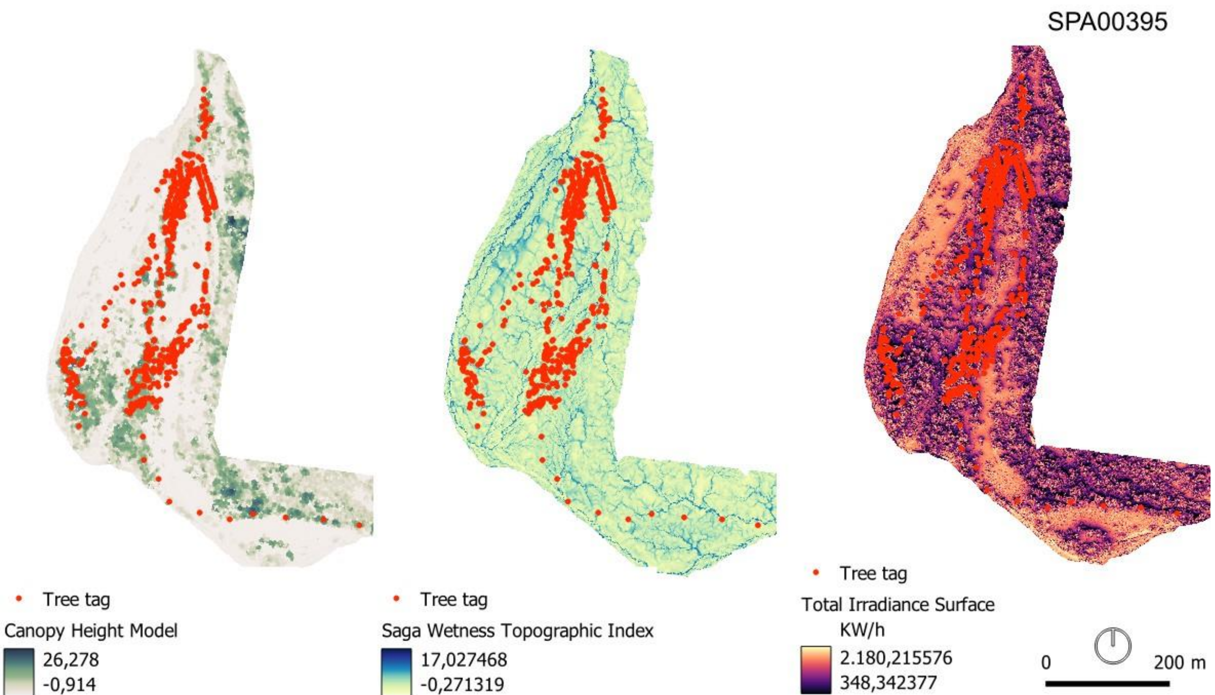


Figure 5 successfully showcases the implementation of an advanced classification method, inspired by the work of Meyer et al. 2022, specifically designed to identify green catkins of *Populus nigra* within the GCU ESP00395. The images highlight the marked catkins, which have been detected with significant accuracy, despite the complexity of the riverine environment and the diversity of textures that pose a challenge for seed classification. Achieving a 79% accuracy rate in our initial training series is a testament to the robustness of our methodology. As previously mentioned, our goal is to extend the application of this model to the entire GCU ESP00395 and to a selected area of AUT00284, captured using RGB images. In December 2023, we plan to make additional adjustments that will allow us to manage the data volume efficiently and further enhance the model's accuracy.

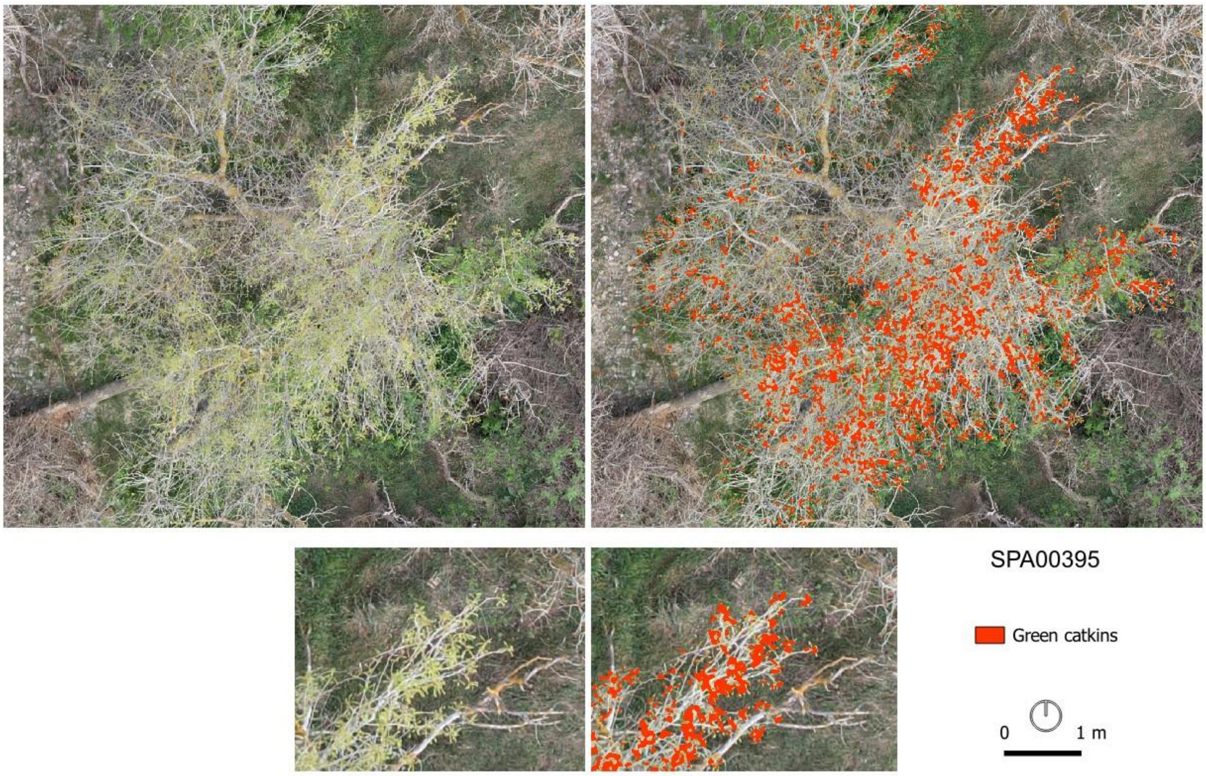


Figure 5. Implementation of an advanced classification method for identifying green catkins of *Populus nigra* within the GCU ESP00395, based on Meyer et al. 2022.

Figure 6 provides the outcomes of a ground-based approach for estimating catkin productivity within the GCU AUT00284. This method, which adapts the workflow described by Goralogia et al. 2021, involves classifying catkins based on their presence and abundance. Utilizing images captured from various angles offers a practical alternative to the logistical and meteorological constraints associated with UAV image capture. Moreover, by comparing this ground-based classification with the automated classification of UAV images, we can assess the effectiveness and accuracy of both methods. This comparative evaluation is crucial for refining our monitoring strategies and enhancing forest management techniques.

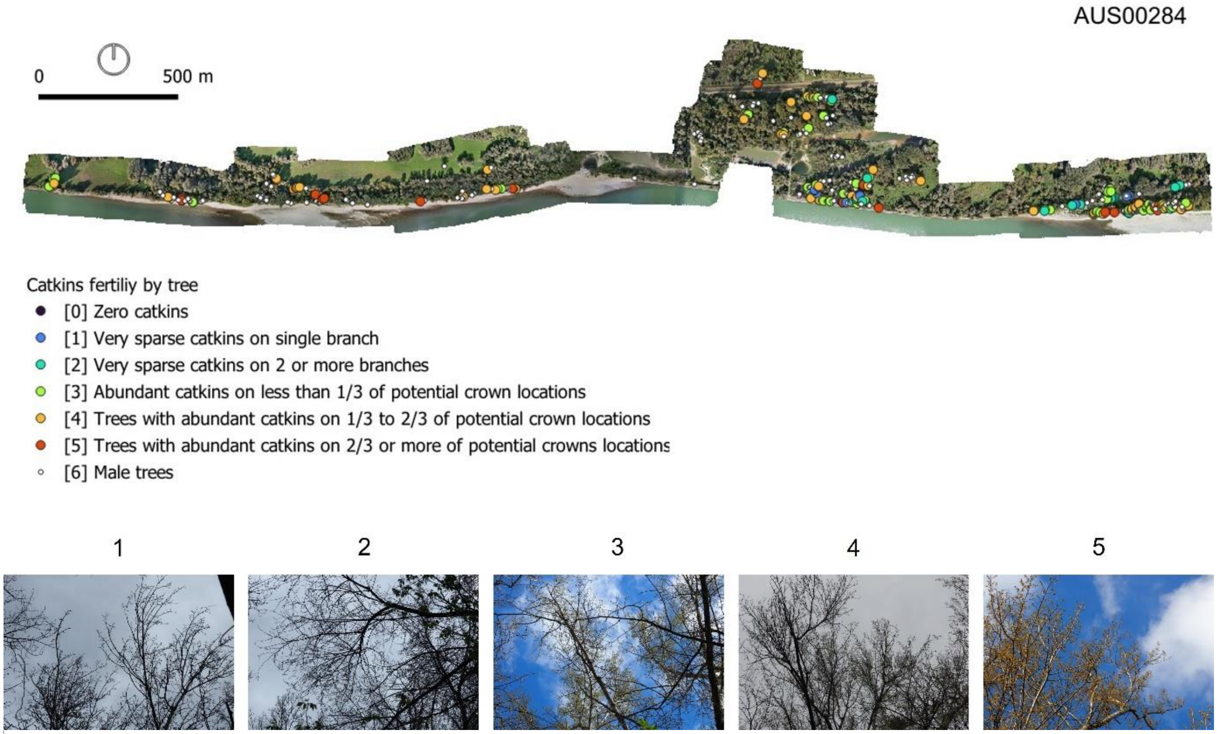


Figure 6. Ground-based catkin productivity assessment within GCU AUT00284, showcasing the classification of catkins by their abundance on *Populus nigra* trees, as adapted from the methodology described by Goralogia et al. 2021.

⇨ All UAV-based measurements are held by UMR.

4 Conclusions

At this stage of the project, we have produced all raw datasets (phenotypic, genetic and environmental data), except for the genomic data and the UAV-based measurements for *Pinus sylvestris* and *Fagus sylvatica*, which will be available within few months. Curated data are being processed adequately, focusing on two species in parallel (*Pinus pinaster* and *Populus nigra*) in order to be able to progress on the methodologies employed in WP3. Now that the full work flow has been tested with these two species (D3.5 and D3.7), it will be easily applied to the remaining species.

5 Partners involved in the work

BFW, CNR, INIA-CSIC, CETEMAS, CITA, INRAE, GIS, Luke, UKCEH, FR, UMR, CREAF

6 Annexes