MIDMACC

Mid-mountain adaptation to climate change

LIFE MIDMACC

Mid-mountain adaptation to climate change

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Executive summary

This deliverable gives an in deep description of the monitoring tasks and network developed to assess and compare the evolution of the pilot experiences in comparison with the initial conditions, with the control plots and with the experiences in other areas of the project. This deliverable explains the monitoring protocol developed for one of the landscape management practices considered in this project, that is, the promotion of mountain agriculture by means of vineyards, both by the conversion of scrubs into vineyards and by the adaptation of agricultural practices to climate change conditions in long-established vineyards, with the purpose of improving the restrictive environmental conditions for agriculture in the mid-altitude Mediterranean mountains. These measures, designed and deployed together with local stakeholders, were implemented by the end of 2019 and beginning 2020 in Aragón and Catalonia. An in deep description of the location of the experiences can be consulted at Nadal-Romero et al (2019, Deliverable 1). The description of the adaptation measures implemented can be consulted at Aranda et al. (2020, Deliverable 7).

The first section is a short introduction to the deliverable, with a briefly description of the pilot experiments and the main objectives of this deliverable. The second section describes the monitoring design implemented in both sites, La Rioja and Catalonia. The third section details the monitoring variables that will be measured in each plot, the frequency of the monitoring and the methodology employed to measure the variables. Finally, the fourth section summarizes all the monitoring tasks implemented in the forest management pilot experiences.

This deliverable presents the activities carried out to monitor the action C3, fundamental in the LIFE MIDMACC project. We have tried to define accurately all the activities that have been carried out this year and that will be carried out in the following four years before evaluating the adaptation measures implemented. We have described all the environmental variables that are going to be measured, with different methodologies, timings, and protocols: (i) soil properties (soil analysis and soil moisture, soil microbial biodiversity), (ii) vineyard production (total production, grape quality, wine quality), (iii) hydrological and sedimentological response (infiltration rates, sediments, times to response), and (iv) site meteorological conditions (air moisture and temperature, rainfall).

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1. Introduction

The main objective of the LIFE MIDMACC project is to promote adaptation to climate change through the implementation and testing of different landscape management measures in mid-mountain areas of Spain: scrubland clearing, forest management and different assays in vineyards in three study areas (Aragon, La Rioja and Catalonia).

The demonstrative activities have been performed in different pilot sites representative of Mediterranean mid-mountain areas. Once the demonstrative activities have been installed, a **monitoring network has been designed, implemented and started**. The objective of the monitoring is to **evaluate the efficiency of the demonstrative activities** to **improve the adaptation capacity to face climate change threatens** and to **improve the socioeconomic development of the mid-mountain areas** where the landscape management measures have been implemented.

In this report, we present the monitoring network related to one of the landscape management practices considered in this project, that is, the promotion of mountain agriculture by means of vineyards (few crops can grow and produce in these conditions and only the vineyard offers a high added value; besides, this also an adaptation measure vineyard to increasing temperatures). Adaptation measures consist both in the conversion of scrubs into vineyards and in the adaptation of agricultural practices to climate change conditions in long-established vineyards, with the purpose of improving the restrictive environmental conditions for agriculture in the mid-altitude Mediterranean mountains. Specifically, the adaptation agricultural practices tested are: use of spontaneous cover crops vs. conventional soil management (tilling, herbicides, mechanical weed removal), use of terraces vs. hillslope cultivation, and vase formation vs. trellising. Most of these adaptations measures were already present in the pilot sites: the specific sites were selected together with local stakeholders according to their own interests.

The **monitoring network implies the establishment and monitoring of a set of permanent monitoring plots and monitoring instrumental** with a triple objective:

- To **assess the adequacy of the actions** implemented to achieve the objective of improving the adaptation capacity to climate change of mid mountain areas agriculture as a means of adaptation to climate change.
- To **evaluate the consequences derived from its application in** maintaining productions and/or improving end product (wine) quality.
- To **assess the effects of these practices on** soil carbon sequestration, soil microbial biodiversity, and hydrological and sedimentological response (infiltration rates, sediments, times to response)

This report presents the monitoring action, a description of the monitoring variables that will be measured in each plot, the frequency of the monitoring and the methodology employed to measure the variables in both regions (La Rioja and Catalonia).

2. Design of the monitoring network

The monitoring network includes the installation of monitoring plots and the design of a protocol for monitoring. The protocol includes a set of variables that will be periodically measured to assess the evolution of pilot experiences, in comparison with plots without landscape intervention (control plots). The monitoring design and the measured variables will be explained in the next sections.

2.1. Monitoring design in La Rioja

The pilot experience has been implemented in Vivanco winery (Tudelilla) and San Prudencio winery (Clavijo), both located in La Rioja. The vineyard pilot experiences encompass a range of conditions and agricultural practices that will be compared among different plots, as terraces vs. hillslope, and both sites (Clavijo and Tudelilla). In total the experience has been implemented in 8 plots covering a total area of 13.24 ha.

- Clavijo (San Prudencio winery). Four areas have been monitored on hillsides and terraces.
    - One plot of 0.2 ha on a terrace has been selected and monitored. One area of this plot will be covered by vegetation (grass), while the other will be ploughed as usual (bare soil).
    - One plot of 0.3 ha (control plot) covered with scrubland on a terrace has been monitored to compare with new vineyards.
    - One plot of 0.8 ha on a hillslope has been selected and monitored. In this case, a small area (0.03 ha) will be covered by vegetation (grass)
    - One plot of 1.03 ha (control plot) covered with scrubland on a hillslope.
- Tudelilla (Dinastía Vivanco winery). Four areas have been monitored on hillsides and terraces with vineyards of different ages. Thus, young, mid-term and old-vineyards have been selected and monitored. The absence and presence of grass cover in the different vineyards will be also evaluated.
    - One plot of 0.74 ha on a small terrace occupied with young vineyard has been monitored.
    - One plot of 6.7 ha on a large hillslope occupied with mid-term vineyard has been monitored.
    - One plot of 1.13 ha on a large hillslope occupied with old vineyards has been monitored.
    - One plot of 2.16 ha (control plot) covered with scrubland has been monitored.

2.2. Monitoring design in Catalonia

The vineyard pilot experiences in Catalonia have been established in three different locations in two different mountain regions: **two sites in Empordà DO** (Roses and Espolla), in the Coastal Pyrenees, and **one site in Llívia, Cerdanya**, in the Central Pyrenees. The latter was not present in the proposal, and was added to the project after first contact with stakeholders, to include a representation of the Central Pyrenees. In total, **the experience was implemented in 9 plots covering a total area of 7.5ha.**

As the adaptive measures, with the partial exception of Llívia, were previous to the project and the pilots were selected to include the selected agronomical practices (crop cover, plant formation, recent transformation to vineyard), the monitoring scheme is included within the pilot design described in Aranda et al. (2020), where a more detailed description of the implemented actions can be found. In summary, the pilot experience consists of:

- **Three plots** were selected in **Espolla**, between 90 and 100m a.s.l.(UTM 31N/ETRS89 coordinates: E(X) 500487.0m N (Y) 4692662.0). Plots have a surface of 1, 0.5 and 1.2 ha, from which a smaller region is monitored (E-C, 0.1ha; E-NC, 0.1 and E-LL, 0.2ha; figure 1, up). Espolla is at Albera massif, in the Eastern Pyrenees, near the coast but 13 km apart from the sea. All three plots are in a plain area with low or no slope. In all three plots vines are of the Carinyena (Carignan) variety grafted on Richter-110. In one of the plots, spontaneous cover crop has been allowed for several years, while in a second plot spontaneous cover has only been allowed from 2019. Finally, in the third plot soil is kept free of vegetation other than grapevines with conventional methods used in the area: tilling or herbicides. With this design, the **impact of an established or new spontaneous cover crop will be compared to the conventional soil management.**
- **Four nearby plots** were selected in **Roses**, in Mas Marés state at about 170 m a.s.l. (UTM 31N/ETRS89 coordinates: E(X) 516783.0m N (Y) 4678347.0m). Plots were chosen as a combination of two factors: trellised vs gobelet ("vas") vines, and hillslope ("coster") vs. terrace plots. The second factor was proposed by plot managers (Espelt winemakers) due to their interest in balancing workforce costs with higher quality. The first factor responds to an interest in **controlling increasing soil erosion in the area**. Plots in Roses are less than a kilometre from the sea, so receiving full sea influence. In all plots, the Garnatxa variety (Grenache, or Lledoner as per its local denomination) is grafted on Richter-110 rootstocks. Plots have a surface of 1.5, 2.3 and 0.1ha, from which a smaller region is monitored (R-T-E, 0.2ha; R-T-V, 0.2ha; R-C-E, 0.1ha and R-C-V, 0.1ha).
- **Two plots** of about 1/3 of ha were selected in **Llívia**, in the Central Pyrenees, in a single estate, at a much higher altitude, 1220 m a.s.l (UTM 31N/ETRS89 coordinates: E(X) 415518.0m N (Y) 4702360.0m). One plot is quite new (right in figures 1 cont. and 4), established as a vineyard in 2012 in a former mare pasture area, and a second plot is newer and has been established in 2020 in an adjacent plot, formerly a cropland. A pastureland plot will be used for some analyses as representative of original conditions of the older vineyard. The original conditions of the newest vineyard have been obtained in 2020 before the establishment of the vineyard. In this pilot, **the short and very short term effects of transformation of pastures or cropland to vineyard will be obtained, which, together with higher altitude, makes this pilot more comparable to La Rioja pilots**.

3. Monitoring variables and protocols

Table 1 summaries the monitored variables in the vineyard pilot experiences in La Rioja and Catalonia. Following, a detailed description of each variable, the means to measure, frequency and specifications is included.

	Variable	Measured variables	Methodology	Periodicity
Soil	**Soil characteristics**	Field bulk density pH and electrical conductivity Total carbon concentration Total nitrogen concentration CN ratio Carbonate content Organic carbon Soil organic carbon and nitrogen stocks Organic matter Organic phosphorus Soil texture Characteristic soil moisture curve (Saturated soil moisture, field capacity and wilting point)	Soil sampling Soil analysis	See Table 2 below
	Soil moisture and temperature	Soil water content (SWC) Soil Temperature (15 cm)	Catalonia: Humidity sensors Teros 10, Teros 11 (Meter) and data-loggers ZL6 (Meter) La Rioja: Humidity sensors S-SMC M 005 humidity probes and data-loggers U30-NRC Meteorological Station HOBO USB	Continuous (2020-2024)
	Soil Microbial Biodiversity	Short, mid and long term effects on soil microbial biodiversity of land use for vineyard establishment. Five replicates per sample. Samples taken end of spring, at veraison.	Soil microbiologic diversity Bacteria and fungus populations: DNA quantitative PCR analysis (bacteria and fungus population size) and DNA semiquantitative Next Generation Sequencing to obtain Biodiversity indices (Shannon, Simpson)	See Table 2 below

Vineyard production	**Total grape production**	Grape Kg per hectare	Information obtained from wine growers and winemakers	Yearly 2020-2023
	Grape Quality	Grape color, Potential Alcoholic strength, pH, Total acidity		
	Wine quality	All relevant parameters according to the Compendium of International Methods of Analysis of Wines and Musts of the Organisation Internationale de la Vigne et du Vin (OIV), such as alcoholic strength, pH, phenolic content… And qualitative value evaluation		
Rainfall simulation	**Hydrological response and soil erosion**	- Runoff coefficient - Infiltration rate - Time to runoff - Ponding time - Wetting front - Sediment concentration - Sediment production - Sediment detachment	Rainfall simulation experiments	Seasonally (at least once in wet and dry soil conditions in each plot)
Site meteorological conditions	**Meteorological variables**	Maximum temperature Minimum temperature Temperature Relative humidity Precipitation Radiation Wind speed	Catalonia: Nearby Meteorological Stations of the Servei Meteorològic de Catalunya (SMC) La Rioja: nearby Meteorological Stations provided by the owners of the Wineries (San Prudencio and Vivanco); Temperature and relative air humidity sensors installed in the plots of the project	Continuous (2020-2024)

Table 1. Summary of the monitored variables in the vineyard pilot experiences in La Rioja and Catalonia.

Site	2020	2021	2022	2023	Total
Llívia	3	1	3	3	10
Empordà Espolla	2	1	0	1	4
Empordà Roses	0	6	0	0	6
La Rioja	0	2	0	0	2

Table 2. Soil samplings for Soil physical and chemical properties and microbiologic diversity estimation along the project in the different sites. All samplings in the same year correspond to a single sampling period (about veraison): number indicates number of plots sampled.

3.1. Soil

Soil is the main reservoir for organic carbon in terrestrial ecosystems. Land uses and land management changes, modified the composition of plant cover, and these changes affect the content and quality of soil properties, especially organic matter and soil organic carbon. The soil organic carbon conservation and its sequestration is of great interest to mitigate the effects of climate change. In that sense, the objective of this environmental monitoring is to identify the effects of vineyard implantation and adaptive agronomic practices to soil properties and soil organic carbon dynamics.

3.1.1. Soil characteristics

The first soil samplings were carried out in July 2020 in all plots in the three locations of Catalonia region. At each monitoring plot, fifteen soil samples were sampled with an auger from 10 to 20 cm. Soil samples were obtained in an X shape centred in the soil moisture sensor location and covering mostly the whole plot. For analysis, samples were grouped in threes according sampling proximity, to create five composite samples per plot. All soil variables listed in Table 1 will be obtained from these samples. Some pilots will be resampled according to Table 2. The analysis will be sent to Eurofins analysis laboratory. In La Rioja, samples will be obtained in mid-2021, together with microbial biodiversity analyses, following the same procedure.

The 2020 analyses will represent long time effects of vineyard establishment in the case of Roses; same for Espolla, for the conventional soil management plot and the long established cover crop plot, and the initial conditions for the newly established cover crop; in the case of Llívia, samples will represent the original conditions for a recently establish vineyard and for a very recently established vineyard, with the nearby pasture representing original conditions.

Some pilots will be resampled according to Table 2 to follow soil evolution under different treatments (for recent or new treatments). For long established treatments (in La Rioja and in some plots in Empordá), no resampling will be performed and data will be compared between plots representing different conditions.

Figure 1. Soil sampling in the control plot (left) and in the long established cover crop (right), in Espolla (Catalonia).

3.1.2. Soil moisture

In La Rioja, a sensor network has been designed to monitor the evolution of soil moisture in the first 45 cm of the soil. The sensor network measures a single profile per plot at one depth (30-40 cm). In each profile, an S-SMC M 005 humidity probe has been installed. These probes are nailed in a vertical position, as recommended in the installation manual, and buried. All sensors are connected to a datalogger that records hourly data. This makes a total of 8 sensors and 4 dataloggers in each site (Clavijo and Tudelilla, total sensors 16 and 8 dataloggers).

Figure 2. Soil moisture sensors and dataloggers in Clavijo (San Prudencio winery), La Rioja, in the hillslope plot (left), terraced plot (middle) and hillslope control plot (right).

Figure 3. Sensors (soil moisture and air temperature and relative humidity) and datalogger in the young vineyard (left) and mid-term vineyard in Tudelilla (Vicanco winery), La Rioja.

Figure 4. Installation of soil humidity sensors and final solar panel in the old-term vineyard in Tudelilla site (Vivanco winery), La Rioja.

In Catalonia, a sensor network has been designed to monitor the evolution of the water in the first 45 cm of the soil, as indicator of water availability for the vegetation and recovery of soil functioning. The sensor network measures a single profile per plot at three different depths: 15, 30 and 45 cm. Each profile site consists in three SWC sensors Teros 10 (Meter), except for one plot per site, were the 15cm Teros 10 is replaced by a Teros 11 sensor which, on top of SWC, measures soil temperature. All sensors are connected to a ZL6 datalogger (Meter) that records hourly data. This makes a total of 9 dataloggers, 24 Teros 10 sensors and 3 Teros 11 sensors.

All dataloggers are connected to a cloud service (Zentra Cloud) to where data are daily uploaded, allowing a close up following of data evolution, malfunctioning early detection, and easy data download, which spares periodic control trips to the different sites.

Figure 5. Soil moisture dataloggers and sensors installed in Roses (left) and Espolla (right)

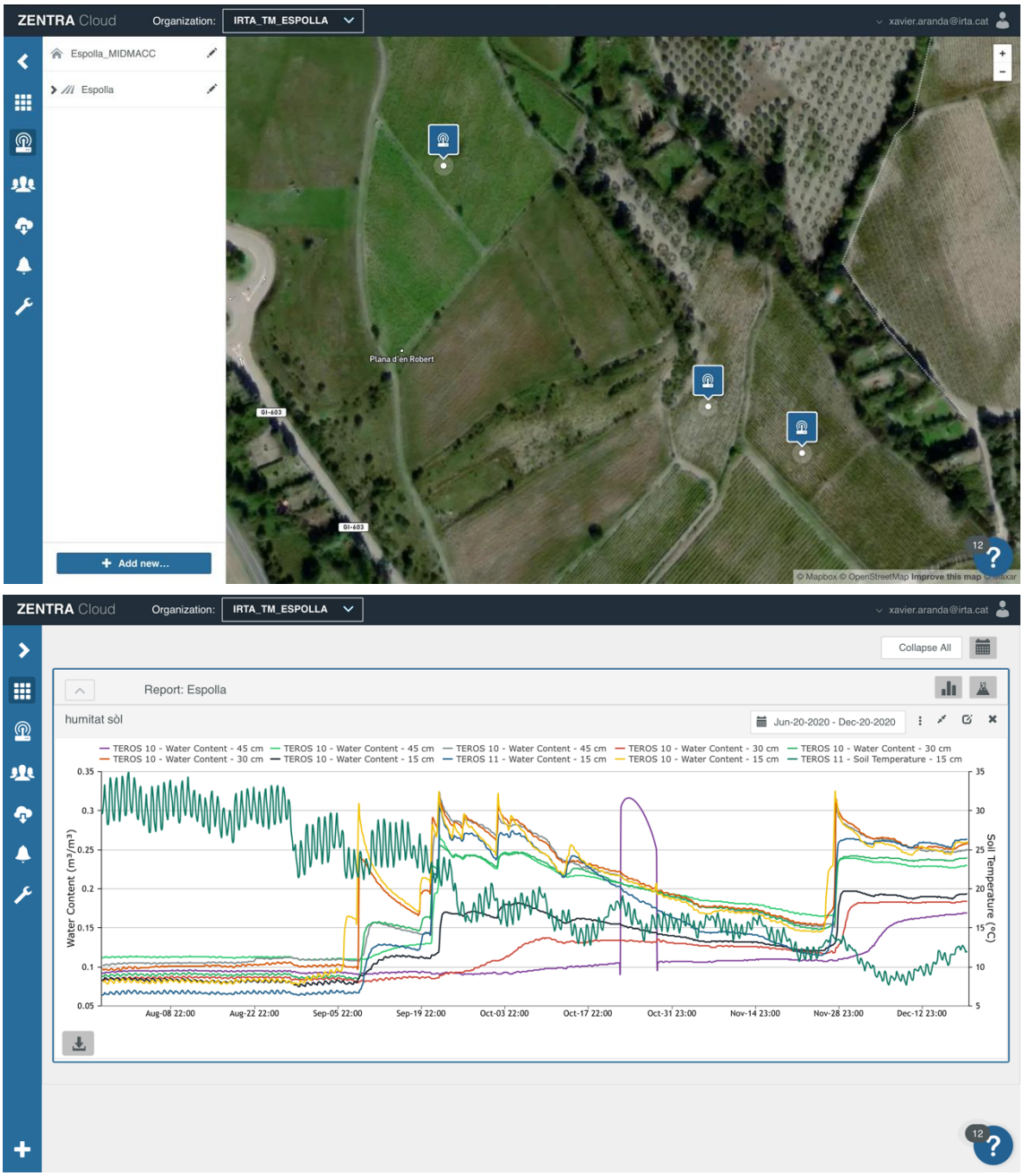

Figure 6. Localization of soil moisture dataloggers and sensors installed in Espolla (top) and data summary (bottom) as presented by Zentra Cloud interface.

3.2. Biodiversity

Soil microbial biodiversity is a solid indicator of soil health. Thus, soil samples obtained at the same time than soil mineral samples have already been collected in July 2020 in Catalonia and will be resampled following the same scheme (Table 2). Soil subsamples (about 5g per sample) are obtained from each sample, preserved in Falcon tubes and transported to the laboratory at 4°C. From each sample, a quantitative DNA PCR analysis of the ribosomal 16S subunit will be performed in IRTA's premises to determine total of bacterial and fungal presence. Samples will also be sent to the University of Illinois for mass sequencing (Next Generation Sequencing, NGS), which will allow to determine the presence of both bacterial and fungal Operational Taxonomic Units, through MiSeq, from which Shanon and Simpson biodiversity indexes will be derived. This allows an assessment of the effects of the different agronomic practices, and of the presence of vineyards, on soil health. In La Rioja samples will be obtained at the in mid-2021 following the same procedure.

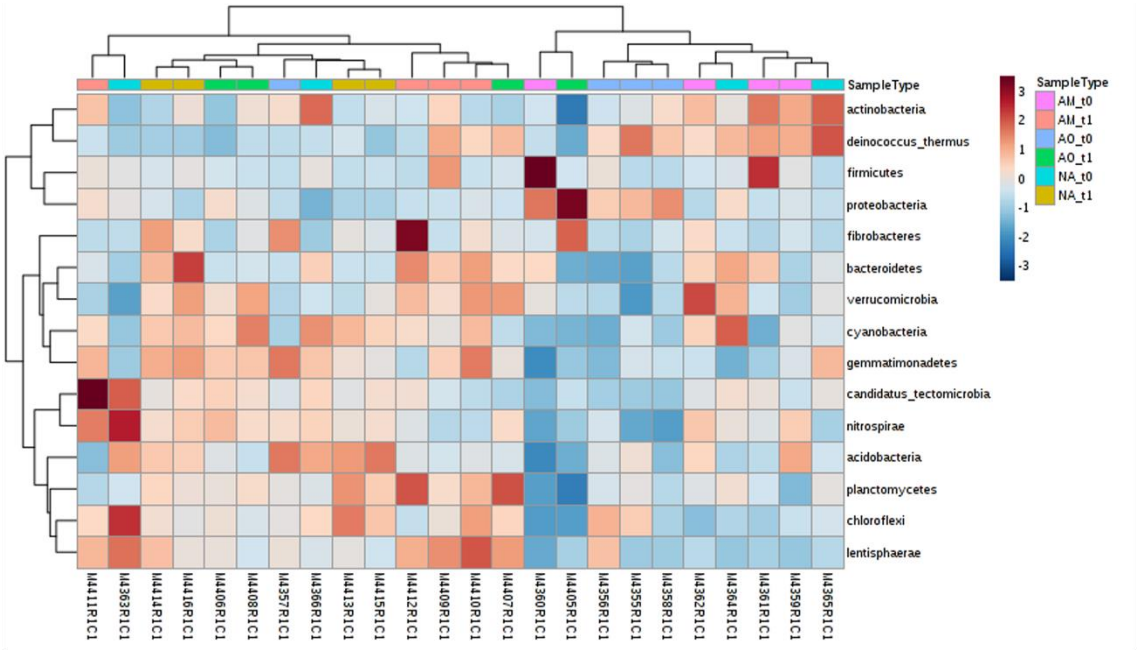

Figure 7. Examples of results of the microbial biodiversity analysis (still ongoing)

3.2.1. Total DNA extraction:

Total DNA is extracted from approx. 0.25 g of soil (n=5 each field test) by using the PowerSoil™ DNeasy Isolation Kit (Qiagen), as a commercial bead beating extraction protocol, according to the instructions of the manufacturer. Total genomic DNA is obtained from triplicate samples.

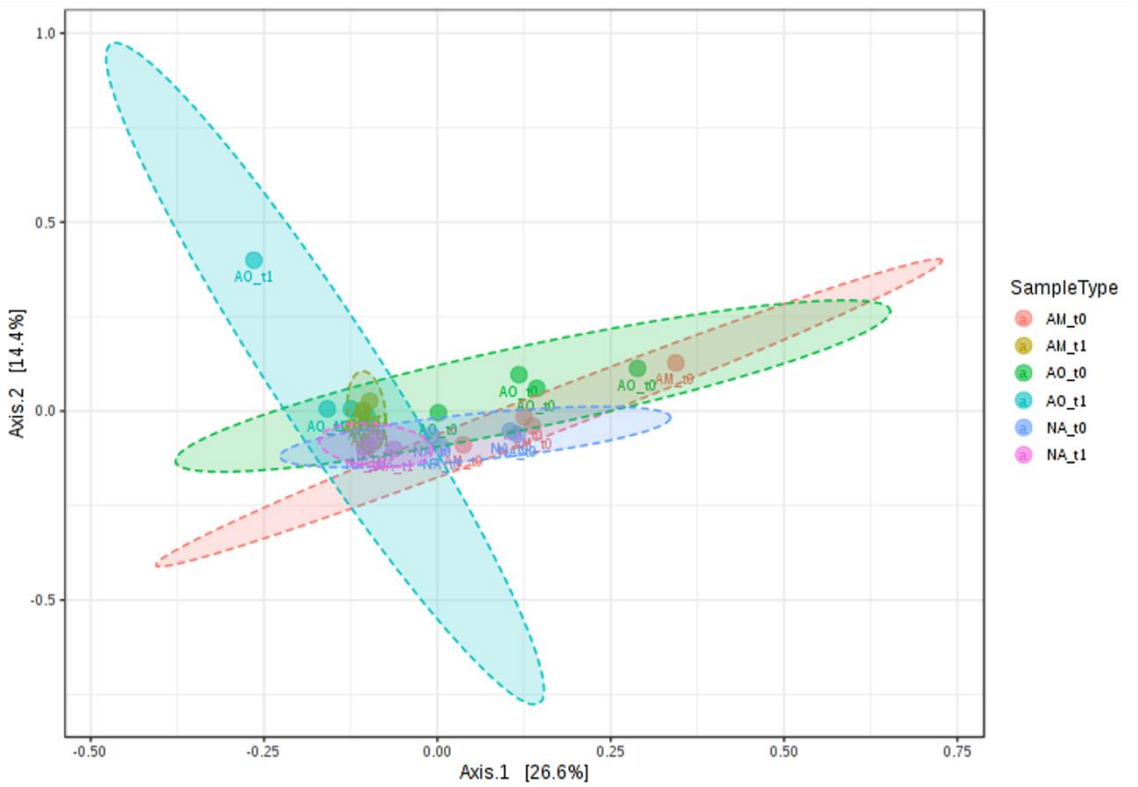

Figure 8.Principal Component Analysis showing the relationships of different treatments and sampling times

Gene copy numbers of total eubacterial 16S rRNA, total fungal community (region ITS1 rRNA) are quantified by means of quantitative real time PCR (qPCR) . Each sample is analysed in triplicate by means of three independent DNA extracts. The analysis is carried out by using Brilliant II SYBR ®Green qPCR Master Mix (Agilent) in a Real Time PCR System MX3000-P (Stratagene, La Jolla, CA).

Total eubacteria (16S rRNA gene) (Yu and Morrison, 2004) and total fungal community (ITS1 region) (White, et al., 1990) is determined by qPCR with the following protocol: 10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 30 s, annealing for 30 s at 50ºC and 55ºC (for 16S rRNA and ITS1 rRNA, respectively), extension at 72°C for 45 s and fluorescence measurement at 80°C. The primer set for eubacterial population is 519FqPCR (5'-GCCAGCAGCCGCGGTAAT-3') and 907RqPCR (5'-CCGTCAATTCCTTTGAGTT-3') (Yu and Morrison, 2004), whereas the primer set for Its1 region are the primers ITS5 and ITS2 (White et al., 1990)

Both analyses are performed by using three independent DNA extracts per sample, by using Brilliant II SYBR ®Green qPCR Master Mix (Agilent) in a Real Time PCR equipment Mx3000PTM (Stratagene). Each reaction is performed in a 12.5 µL volume containing 1 µL of DNA template, 200 nM of each primer, 6.25 µL of the ready reaction mix and 30 nM of ROX reference dye. The qPCR programme is set as follows: 10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 30 s, annealing for 30 s at 50ºC and 62ºC (for 16S rRNA and nosZ gene, respectively), extension at 72°C for 45 s and image capture at 80°C.

The specificity of PCR amplifications for all studied gene (16S rRNA, ITS1, and nosZ) is determined by observations on the melting curve and gel electrophoresis profile. Melting curve analysis to detect the presence of primer dimers is performed after the final extension PCR step by increasing the temperature from 55 to 95°C in 0.5°C/10s. Fluorescence acquisition step is performed at 80°C to exclude fluorescence from the amplification of primer dimers. Serial dilutions of total DNA extracts from soil matrix and groundwater are quantified and compared to check for the potential presence of PCR inhibitors.

To perform calibration curves, standards are prepared based on the following reference genes: 16S rRNA gene from Desulfovibrio vulgaris subsp. Vulgaris ATCC 29579, inserted in a TOPO TA vector (Invitrogen, Merelbeke, Belgium). The primer pairs 519f (5'_ GCC AGC AGC CGC GGT AAT_3') and 907r (5'_CCG TCA ATT CCT TTG AGT TT_3'), as proposed by Lane (1991) to amplify 388 pb of the 16S rRNA gene. ITS1 gene fragment are obtained from a single DGGE band (genbank accession nr JN982550) cloned onto the PGEM plasmid vector using PGEM-T Easy Vector System I (Promega, Madison, WI, USA).

All reference genes are initially quantified by NanoDrop 1000 (Thermo Scientific). Ten-fold serial dilutions of known copy numbers of the plasmid DNA in the range 10 to 108 copies are subjected to a qPCR assay in duplicate to generate the standard curves. All primer sets are purified by HPLC and all reference genes are quantified by Nanodrop 1000 and calculated using the following formula:

$$\textit{Gene copies}/\mu L = \frac{\frac{ng\ DNA}{\mu L} \times \frac{1\ g}{10^9\ ng} \times \frac{1\ mol\ pb}{660\ g\ DNA} \times \frac{6.022^{23}\ pb}{1\ mol\ pb} \times \frac{1\ copy}{plasmid\ size + insert\ (pb)}}{\frac{ng\ DNA}{\mu L} \times \frac{1\ g}{10^9\ ng} \times \frac{1\ mol\ pb}{660\ g\ DNA} \times \frac{6.022^{23}\ pb}{1\ mol\ pb} \times \frac{1\ copy}{plasmid\ size + insert\ (pb)}}$$

To generate every single standard curve ten-fold serial dilutions of the known copy number of each plasmid DNA are subjected to a qPCR assay in duplicate. All the analyses are previously optimized by performing inhibition assays: the samples are diluted at different concentrations: 1, 1:10 and 1:100. Taking into account the Ct values and the detection limit the selected dilution is properly prepared.

The qPCR efficiencies of amplification are greater than 98%. All results are processed by MxPro QPCR Software (Stratagene).

3.2.2. NGS sequencing of 16SrRNA eubacterial, ITS2 region (Fungi), and V4-18S region (microalgae) populations by means MiSeq platform

Massive tagged 16S rRNA gene libraries targeting eubacterial region V3-V4 16S rRNA and fungal region ITS2 (5,8S-ITS2-28S), are sequenced utilizing MiSeq equipment (Illumina). In summary, diluted DNA extracts (1:10) are utilized as a template for PCR. Each DNA (three independent total DNA extract per sample) are amplified separately with both V3-V4 16S rRNA eubacteria, ITS2 fungal region, and V4-18S for microalgae , by using set of primers containing unique multiplex identifier (MID) tags. For eubacteria libraries the primer set are V3_F357_N: 5'-CCTACGGGNGGCWGCAG and V4_R805: 5'-GACTACHVGGGTATCTAATCC, while fungal community (ITS3: 5′-GCATCGATGAAGAACGCAGC-3′ ITS4: 5′-TCCTCCGCTTATTGATATGC) for region ITS2 (White et al., 1990; Bellemain et al., 2010) will be utilized.

A single-step 30 cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) is run following next conditions: 94ºC for 3 minutes, followed by 28 cycles of 94ºC for 30 seconds; 53ºC for 40 seconds and 72ºC for 1 minute; and a final elongation step at 72ºC for 5 minutes. All PCR products from different samples are mixed in equal concentrations by using Quant-iT PicoGreen dsDNA Kit (Invitrogen, Carlsbad, CA, USA). Purification is performed by using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). DNAs are sequenced utilizing MiSeq (Illumina, 2x300 bp kit) instrument following manufacturer's guidelines at Molecular Research DNA facilities.

Downstream MiSeq data analysis iss carried out by using QIIME software version 1.8.0 The obtained DNA reads are compiled in FASTq files for further bioinformatic processing. Trimming of the 16S rRNA barcoded sequences into libraries is carried out using QIIME2 software (Caporaso et al., 2010). Quality filtering of the reads is performed at Q25, prior to the grouping into OUT/Amplicon Sequence Variants (ASV) at a 99% sequence homology cutoff. The following steps are performed using QIIME: Denoising using Denoiser [Reeder and Knight, 2010) and DADA2. Reference sequences for each OTU (OTU picking up) are obtained via the first method of UCLUST algorithm (Edgar, R.C., 2010). For sequence alignment is used PyNAST (Caporaso et al., 2010b) and Chimera detection is used ChimeraSlayer (Haas et al., 2011). ASVs are then taxonomically classified using BLASTn against GreenGenes and RDP (Bayesian Classifier) database and compiled into each taxonomic level (DeSantis, Hugenholtz et al. 2006). Mothur/Qiime/Microbiome Analyst software is utilized to determine alpha-diversity indices, rarefaction curves and beta diversity assessment.

Data from MiSEq is submitted in the meantime to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) for further scientific publication at SCI indexed journal.

All statistical analysis are performed by means Mothur (Schloss et al., 2009) the Microbiome Analyst Software (Chong et al., 2020) and are described below.

3.2.3. Alpha diversity analysis of Microbial communities

This method is used to measure the diversity present within a sample or community. Alpha diversity can be characterized via the total number of species or OTUS/ASV in one sample (richness), the abundances of the species (evenness) or measures that considered both richness and evenness (diversity indexes such as Shannon (H) and Inverted Simpson (1/S). How these measures estimate the diversity is need to be considered when performing alpha-diversity analysis. The richness is estimated by Chao1 by inferring out the number of rare organisms that may have lost due to undersampling. Alpha diversity analysis are performed by using the Phyloseq R package and Mothur software (1.44.3), and data are rarefy to the minimum reads observed in the whole set of samples.

3.2.4. Beta diversity Analysis of Microbial communities

This method provides a way to compare the diversity or composition between two samples or microbial communities. These methods compare the changes in the presence/absence or abundance of thousands of taxa present in a dataset and summarize these into how 'similar' or 'dissimilar' two samples. Each normalized sample (Total Sum Scaling (TSS) of OTU/ASV) gets compared to every other sample generating a distance or dissimilarity matrix. The parameter considered when performing beta diversity analysis is similarity or distance between sample measured based on non-

phylogenetic-based distances of Total Sum Scaling normalized OTUs abundance (Bray-Curtis distance). The other parameter is used to visualize such dissimilarity matrix in lower dimensions. Ordination-based methods such as Principle Coordinate Analysis (PCoA) are used to visualize these matrix in 2-D plot where each point represents the entire microbiome of a single sample. Each axis reflects the percent of the variation between the samples with the X-axis representing the highest dimension of variation and the Y-axis representing the second highest dimension of variation. Further, each point or sample displayed on PCoA plots is coloured based on either sample group, features alpha diversity measures, or the abundance levels of a specific feature.

Also, the statistical significance of the clustering pattern in ordination plots are evaluated using anyone among Permutational ANOVA (PERMANOVA). PERMANOVA formulates the null hypothesis of no differences in composition among groups under the condition that the different groups of samples have the same centres (centroids). The variability within groups is compared against the variability between groups with ANOVA F statistic (but portion of sums-of-squares is applied directly to dissimilarities).

Beta diversity analysis are performed using the R phyloseq package run by means of Microbiome Analyst software.

3.3. Vineyard production

Central to the vineyard pilot experiences is to determine if adaptation of mid mountain to climate change can be achieved through agriculture and more specifically through vineyard establishment. Complementarily, the feasibility of vineyard migration to mid mountain as an adaptation measure of the vineyard to climate change will also be assessed. To answer both points, grape production per hectare, grape quality and, most significantly, wine quality will be studied. As vineyard pilots are completely governed by local stakeholders (wine growers, winemakers), data will be yearly obtained from them. Adaptation criteria have not been imposed to local stakeholders, which means they may slightly differ from one site to another: conserving total production, wine quality or both, or obtaining new wine profiles, such as ice wine or different aromas may be two different strategies of adaptation, both for local wine growers and for winemakers of other parts of Catalonia.

This specificity makes it difficult to list specific parameters to be tested: beyond obligatory analyses mandated by law, the sensorial and wine definition parameters are unique to each winemaker, and form part of their commercial strategies. Hence, we will rely on their own definitions to assess the adaptation potential both of vineyard establishment in the mid mountain and of the specific agronomic practices.

3.4. Rainfall simulations

Land use and land cover determines the relationship between precipitation and both runoff and soil erosion. The establishment of a vineyard, whether recent or long time, affects the vegetation cover, which in turn affects interception and evapotranspiration of the plants, and also the soil properties, with significant consequences for runoff and soil erosion. The use of different soil management practices, such as tilling, implementation of cover crops, terraces and others, will result in quite different hydrogeomorphological effects. The objective of this environmental monitoring is to assess the effect of vineyard establishment or the use of adaptive agronomic practices on the hydrological response and soil erosion.

For this purpose, we will carry out rainfall simulation experiments seasonally in all monitoring plots, at least once in dry soil conditions and in wet soil conditions. Rainfall simulations are widely used to compare and assess runoff and sediment production by rain splash because they enable initial conditions to be established and provide for control over rainfall characteristics (Iserloh *et al*., 2012). We will use a portable rainfall simulator designed for rugged terrain (Figure 18). The simulator consists of a metallic structure with telescopic metal legs, and is covered with plastic to protect the experiments from wind. On the top of the structure a nozzle is installed. In our experiments, we use a rainfall intensity ranging from 30 to 45 mm h^{-1}, which corresponds to a moderate-to-high rainfall intensity event. Rainfall is registered in each experiment with two pluviometers. The experimental plots are defined by a circular ring with an area of 0.25 m^2. Each plot has a drain pipe outlet for collection of runoff samples (Figure 19), located down slope at surface level. In each experiment, several variables characterizing the hydrogeological and sedimentological response are obtained: Runoff coefficient (%), Infiltration rate (mm h^{-1}), Time to runoff (min), Ponding time (min), Wetting front (cm), Sediment concentration (mg l^{-1}), Sediment production (g) and Sediment detachment (g m^{-2} h^{-1}).

The first experiments were carried at in October-November 2020: in Catalonia, in the three sites in all plots plus some more control plots (non-vineyard sites) (Figure 9, Figure 10); and in La Rioja in all plots except the ones with vegetation cover as the grass had not grown yet (Figure 11).

Figure 9. The rainfall simulator used during the experiments in Llívia (Catalonia): general view (left), simulator at work (right).

Figure 10. Collecting water samples during a rainfall simulation in Roses (Catalonia).

Figure 11. Installing the circular ring in Tudelilla (left) and overview of the rainfall simulator in a control plot in Clavijo (right), La Rioja.

3.5. Site meteorological conditions

The registration of the meteorological conditions is key to understand the evolution of previous variables along the project duration.

In La Rioja, temperature and moisture air sensors have been installed in both locations (Figure 12). Rainfall data will be analysed by nearby meteorological stations provided by the owners of the Winery (San Prudencio and Vivanco wineries).

Figure 12. Air temperature and relative humidity sensors HOBO Logger installed in (left) Clavijo site (San Prudencio winery) and (right) Tudelilla site (Vivanco winery), La Rioja.

In Catalonia plots, as all three sites have nearby meteorological stations of the SMC (Servei Meteorològic de Catalunya), AEMET (Agencia Estatal de Meteorología) or Météo-France, sometimes adjacent to the plots. This publicly available information will be used (Table 3).

Site	Reference Meteorological Station
Espolla	Espolla (SMC)
Roses	Roses (SMC)
Llivia	Martinet (AEMET) / Sainte Léocadie (Météo-France)
Clavijo	San Prudencio winery
Tudelilla	Vivanco winery

Table 3. Reference meteorological station per experimental pot.

4. Conclusions

The main objective of this deliverable is to present the **design of the monitoring network and describe the monitoring variables and protocols** of the action C.3: Climate change adaptation measure: Assays and experiences in vineyards in A Rioja and Catalonia.

Table 1 summarizes all the variables, briefly describes methods, and indicates the periodicity of the different monitoring protocols. Physical and chemical soil properties and soil moisture, soil microbial biodiversity; vineyard production and quality; rainfall simulations and meteorological conditions are going to be measure to analyse the effects of adaptive vineyard establishment and agronomic practices.

Finally, it should be highlighted that the **all the monitoring variables have been already measured at the beginning of the implementation activity (2020)** and data are going to be analysed by different project partners. Consequently, **all the activities and the periodicity defined in the proposal have been successfully completed** and it is a perfect starting point for the future monitoring network defined in the LIFE MIDMACC project.

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