LIFE MIDMACC

Mid-mountain adaptation to climate change

LIFE18 CCA/ES/001099

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Executive summary

This deliverable presents the results obtained from monitoring of the pilot experiences during the activities carried out up to December 2023 to monitor the action C3. The pilot experiences were implemented by the end of 2019 and beginning of 2020 in Catalonia and La Rioja. The setting of monitoring variables was performed from middle 2020, the first complete monitoring campaign was carried out in 2021, and the second in 2022. Following the monitoring protocol explained in Deliverable 10 (Aranda et al. 2020), this document presents the second-year results obtained in the pilot experiences in vineyards in La Rioja and Catalonia.

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 summarises the monitoring protocol, to have a quick overview of the monitored variables in Catalonia and La Rioja. The third and fourth sections detail the preliminary results of the variables measured in both sites of Catalonia and La Rioja during the three campaigns. Finally, the fifth section summarizes the main outcomes found up to this moment.

We have tried to define accurately all the activities that have been carried out this first and second year and that will be carried out in the following years before evaluating the adaptation measures implemented. We have presented the preliminary results of all the environmental variables that are been measuring and also all the 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).

Mid-mountain adaptation to climate change

With the support of

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Content

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 preliminary results of 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 could be also considered as an adaptation measure to grow grapevine under global warm scenario). 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 adaptative 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. trellis. Most of these adaptation 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 subsequent 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).

2. Summary of the monitoring protocol

Deliverable 10 (Aranda et al., 2020) collects all aspects related with the monitoring of pilot experiences and summaries the monitored variables in the vineyard pilot experiences in Catalonia and La Rioja. A detailed description of each variable, the means to measure, frequency and specifications can be consulted at Aranda et al., 2020.

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

	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 Diversity	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 microbial diversity of Bacterial and fungal populations: DNA quantitative PCR analysis (bacterial and fungal populations size) and DNA metabarcoding by paired end amplicon sequencing (Metabarcoding of 16S rRNA and ITS2 region) of bacterial and fungal communities to obtain alpha and beta diversity indices (Chao1, Shannon, inverted Simpson and PCoA-Bray Curtis)	See Table 2 below

	Variable	Measured variables	Methodology	Periodicity
Vineyard production	**Total grape production**	Grape Kg per hectare	Information obtained from wine growers and winemakers	Yearly 2020-2023
	Grape Quality	Grape colour, 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 (2020 as a wet soil, 2021 as a dry soil and also 2022 as a wet soil condition in each plot)
Site meteorologica l conditions	**Meteorologic al variables**	Maximum temperature Minimum temperature Temperature Relative humidity Precipitation Wind speed	Catalonia: Nearby Meteorological Stations of the Servei Meteorològic de Catalunya (SMC) and ICGC 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)

3. Preliminary results of the monitoring campaigns in vineyards in Catalonia

3.1. Monitoring results of the Soil

3.1.1. Soil characteristics.

The first soil samplings were carried out in July 2020 and 2021 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 three according to sampling proximity, to create five composite samples per plot. Llívia (CAT) pilot has been resampled in 2023 (Table 2). The 2020, 2021 and 2023 analysis were sent to Eurofins analysis laboratory, and results are presented in Figures 1, 2a, 2b & 3.

Table 2. *Soil samplings for soil physical and chemical properties and microbial diversity monitoring 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. *Extra sampling in Espolla due to soil texture inconsistencies.*

Site	2020	2021	2022	2023	Total
Llívia (CAT)	3	0	0	3	6
Empordà Espolla (CAT)	3*	0	0	0	3*
Empordà Roses (CAT)	0	5	0	0	5

The 2020 analyses represent long time effects of vineyard establishment in the case of Espolla, for the conventional soil management plot (CM) and the long-established cover crop plot (WE), and the initial conditions for the newly established cover crop (NC), with the nearby fennel (F) representing original conditions; in the case of Llívia, samples represent the original conditions for a recently establish vineyard (NV) and for a very recently established vineyard (VNV), with the nearby pasture (P) representing original conditions. In Roses case, 2021 samples represent terraces vs. hillslope cultivation, and vase formation vs. trellis. The Roses plots are Trellis slope (TS), Trellis Terrace (TT), Gobelet Terrace (GT), Gobelet Slope (GS) and Scrubland (S).

Llívia (CAT) plot was resampled in 2023 (Table 2) to follow the soil evolution under different treatments (for recent or new treatments). For long established treatments, no resampling will be performed, and data will be compared between plots representing different conditions. The 2020 soil results analyses are shown in Figure 1 (Espolla), Figure 2a (Llívia) and for 2021 in Figure 3 (Roses). The 2023 results of Llívia (CAT) plot are shown in Figure 2b.

In Espolla, NC plot (Espolla) shows higher values of macronutrients (N-P-K), organic carbon and organic matter than WE and CM plots. In general, NC soils (spontaneous cover has only been allowed from 2019) have high values of macronutrients and organic matter (Figure 1). Therefore, the soil impact in terms of nutrients availability of a new spontaneous cover crop is lower than a soil conventional management, which presents lower values of these nutrients. In the case of a spontaneous cover crop allowed for several years (WE), the behaviour is quite similar than CM (Figure 1).

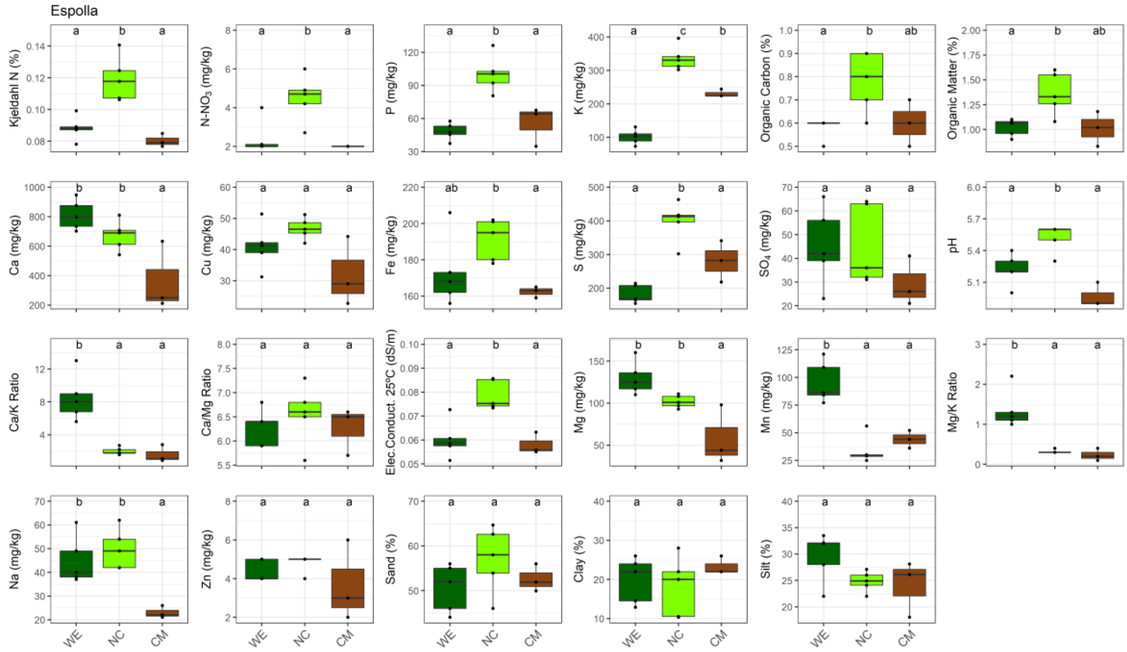


Figure 1*. Box plot of soil characteristics variables in Espolla plot. Dark green boxes represent Well Established Cover (WE), light green boxes represent New Cover (NC) and brown boxes represent Conventional Soil Management (CM). The box portion of the box plot is defined by two lines at the 25th percentile and 75th percentile. The two whisker boundaries are the 5th and 95th percentile. Lowercase letters indicate significant differences between plots ($p<0.05$).*

In Llívia, VNV showed higher macronutrients than NV, but similar organic carbon and organic matter (Figure 2a). As expected in a cattle grazing, pastureland showed higher values of organic carbon, organic matter, and N. Therefore, the short and very short-term effects of transformation of pastures to vineyard are decreases in organic matter, organic carbon, and nitrogen soil availability, and for contrary, increases of P, K and N-NO_3 in the soil. In general, small differences have been observed between 2020 and 2023 in terms of soil characteristics soil results in Llívia plot. NV and VNV showed higher P and K in 2023 than in 2020. Additionally, VNV showed an increase in Mn in 2023. In contrast, Pasture do not show any difference between both years (Figure 2b). The absence of substantial differences between years could be explained by the short time of actions (2/3 years). Generally, the effects of actions performed to the soil are often cumulative, and the full benefits only become more apparent over time.

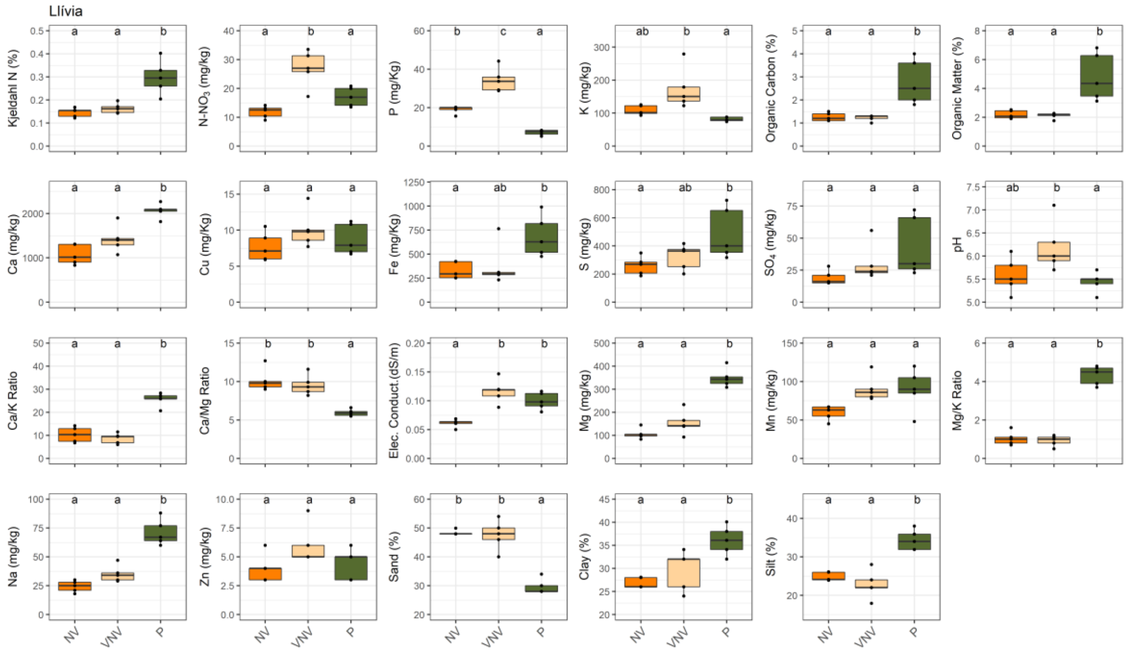


Figure 2a. *Box plot of soil characteristics variables in Llívia plot. Orange boxes represent New Vineyard (NV), beige boxes represent Very New Vineyard (VNV) and dark green boxes represent Pasture (P). The box portion of the box plot is defined by two lines at the 25th percentile and 75th percentile. The two whisker boundaries are the 5th and 95th percentile. Lowercase letters indicate significant differences between plots (p<0.05).*

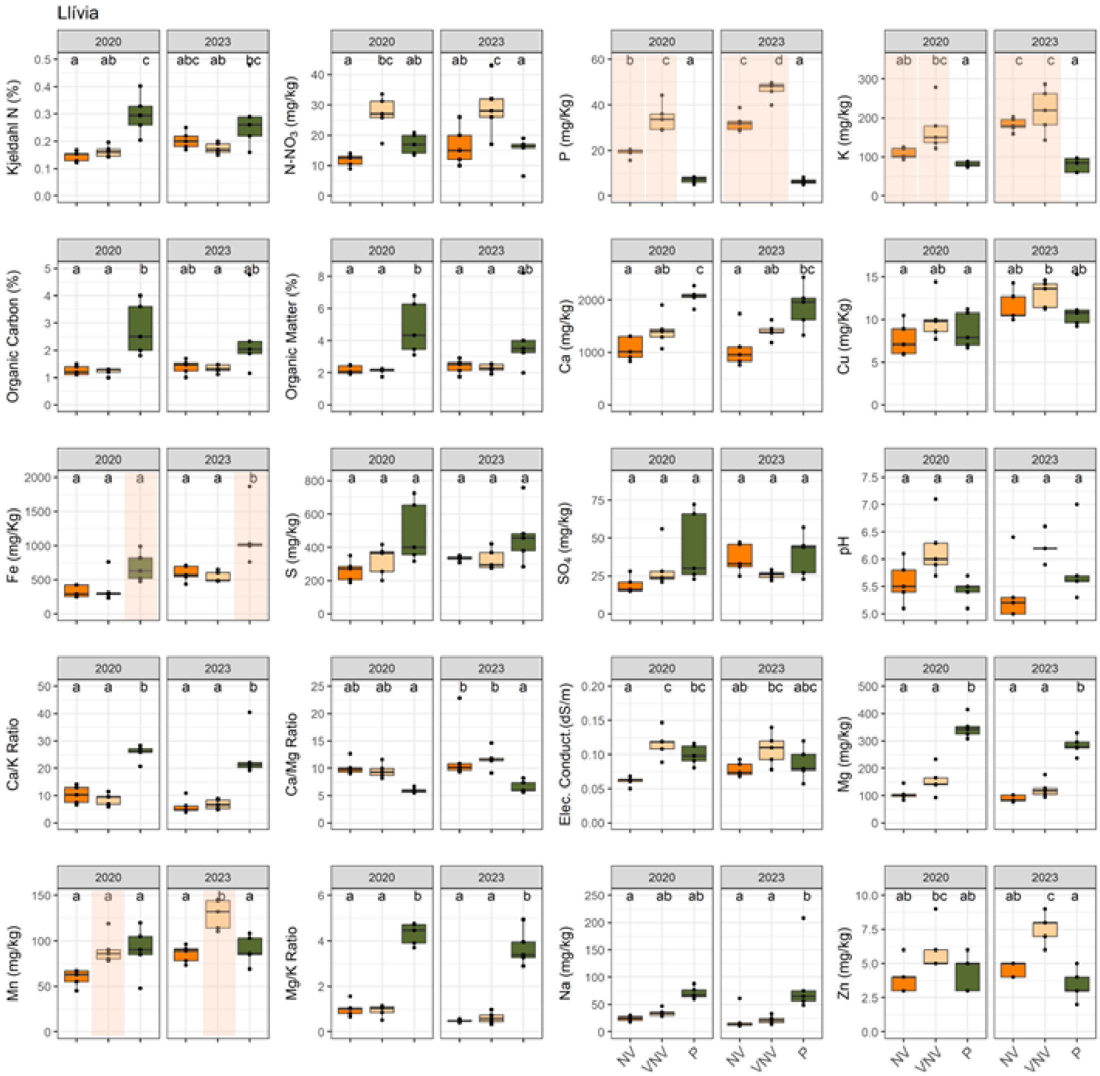


Figure 2b. *Comparative box plot of soil characteristics variables in Llívia plot for 2020 and 2023. Orange boxes represent New Vineyard (NV), beige boxes represent Very New Vineyard (VNV) and dark green boxes represent Pasture (P). The box portion of the box plot is defined by two lines at the 25th percentile and 75th percentile. The two whisker boundaries are the 5th and 95th percentile. Lowercase letters indicate significant differences between plots ($p<0.05$). The shaded orange areas highlight the differences between years for some parameters.*

In Roses, there are no differences between the adaptation measures (terraces vs. hillslope cultivation, and vase formation vs. trellising) in terms of macronutrients, except for GT for Kjeldahl nitrogen, with lower values than the rest of the plots. Organic carbon and organic matter are also lower in GT than the other plots. For scrubland, considered as the control, the values of Cu and SO_4 are lower, and for the contrary, the values of Na are higher than the rest of the plots. In TS the values of Mg, SO_4 and Na are lower (Figure 3).

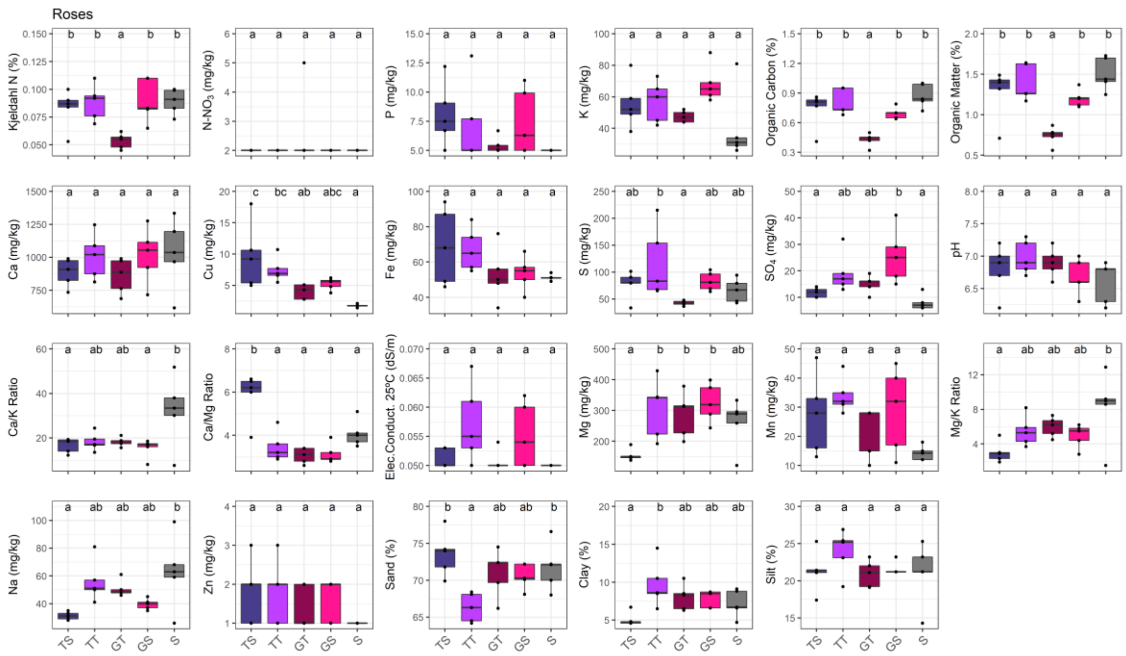


Figure 3. *Box plot of soil characteristics variables in Roses plot. Dark purple boxes represent Trellis Slope (TS), blueviolet boxes represent Trellis Terrace (TT), orange red boxes represent Gobelet Terrace (GT), pink boxes represent Gobelet Slope (GS) and grey boxes represent Scrubland (S). The box portion of the box plot is defined by two lines at the 25th percentile and 75th percentile. The two whisker boundaries are the 5th and 95th percentile. Lowercase letters indicate significant differences between plots ($p<0.05$).*

In order to understand differences among the three plots (e.g. Espolla, Llívia and Roses), a PCA was performed (figure 4). Results showed a separation at the first component (≈43%) of Roses in relation to the other two plots. Espolla and Llívia in turns were merely separated at the second component. However, the pasture treatment in Llívia (the both years) separated at the second component from all the rest of treatments, confirming the richest values of fertility components (such as organic matter, organic carbon, nitrogen among others).

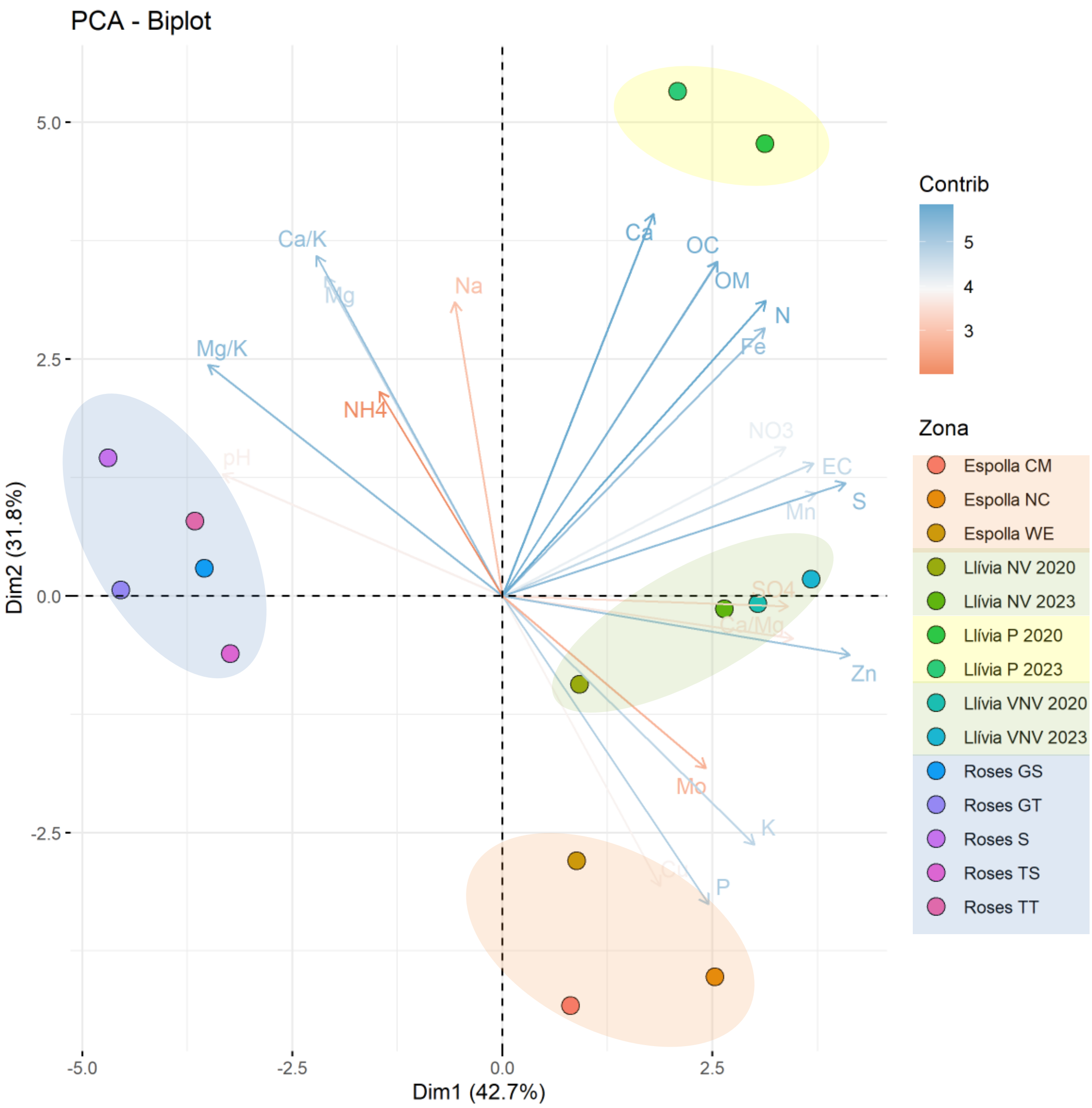


Figure 4. *PCA Biplot. OM: organic matter, OC: organic carbon; EC: Electrical conductivity*

Another analysed variable is soil texture, which is in general, sandy loam and sandy clay loam for all Espolla plots, sandy clay loam for Llívia vineyards, except in pasture (P) which is clay loam, and Sandy Loam for Roses plots (Figure 5).

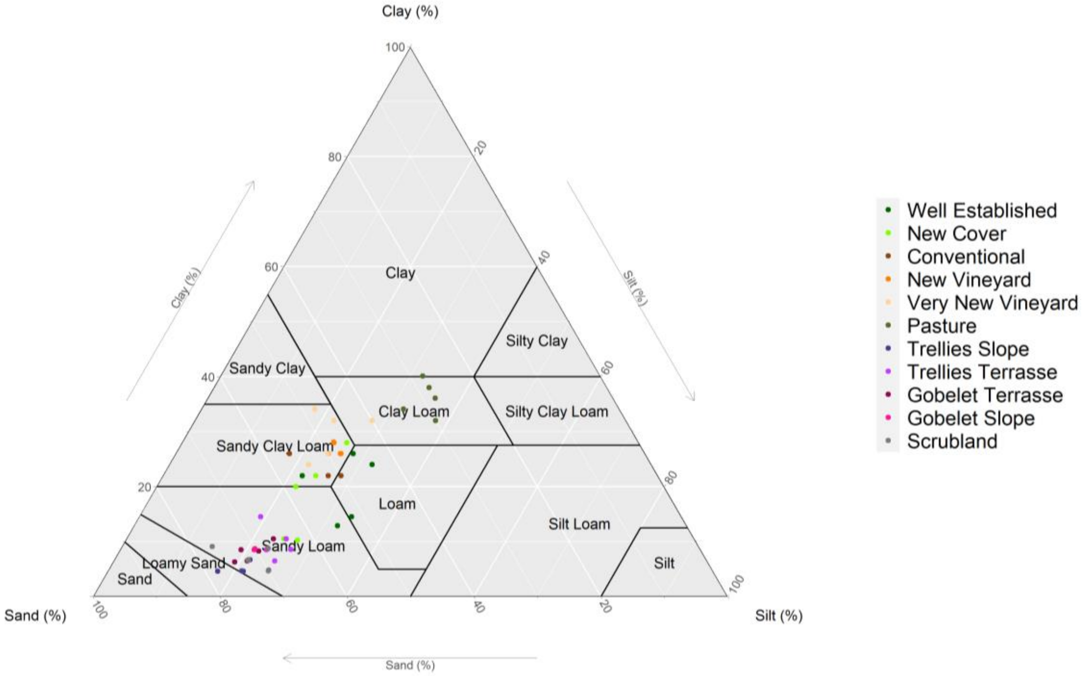


Figure 5. *Soil texture triangle with all the samples from Espolla, Llívia and Roses plots. Different colours represent the different agricultural practices.*

3.1.2. Soil moisture

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 measured in continuum from August 2020 to the present (here we present the results until December 2023) (Figures 6, 7 and 8).

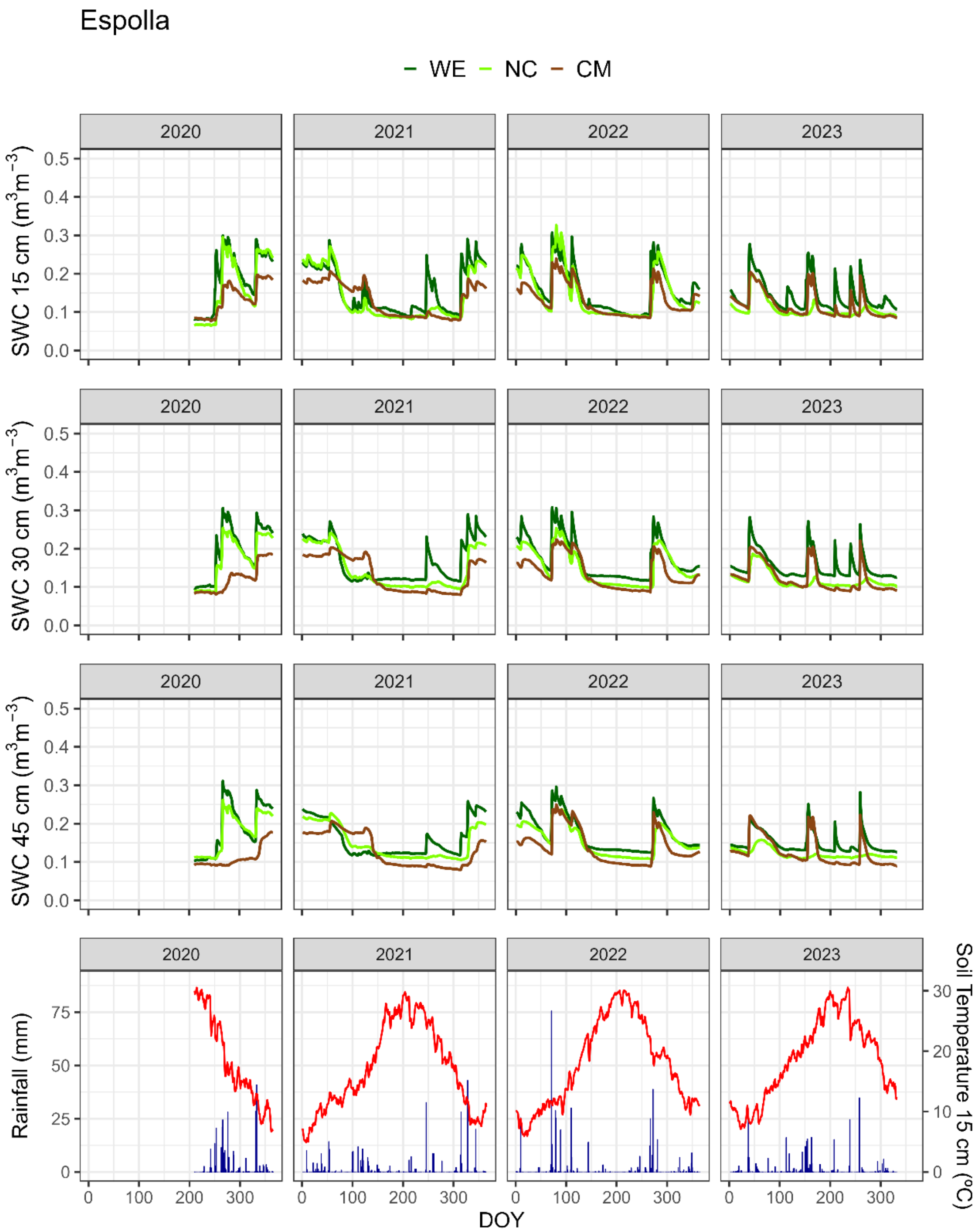


Figure 6. *Daily soil water content evolution in Espolla pilot from August 2020 to December 2023. Each rectangle shows different sensor depth (15, 30 and 45 cm) and rainfall and soil temperature at 15 cm. Legend colour are the different agronomical practices.*

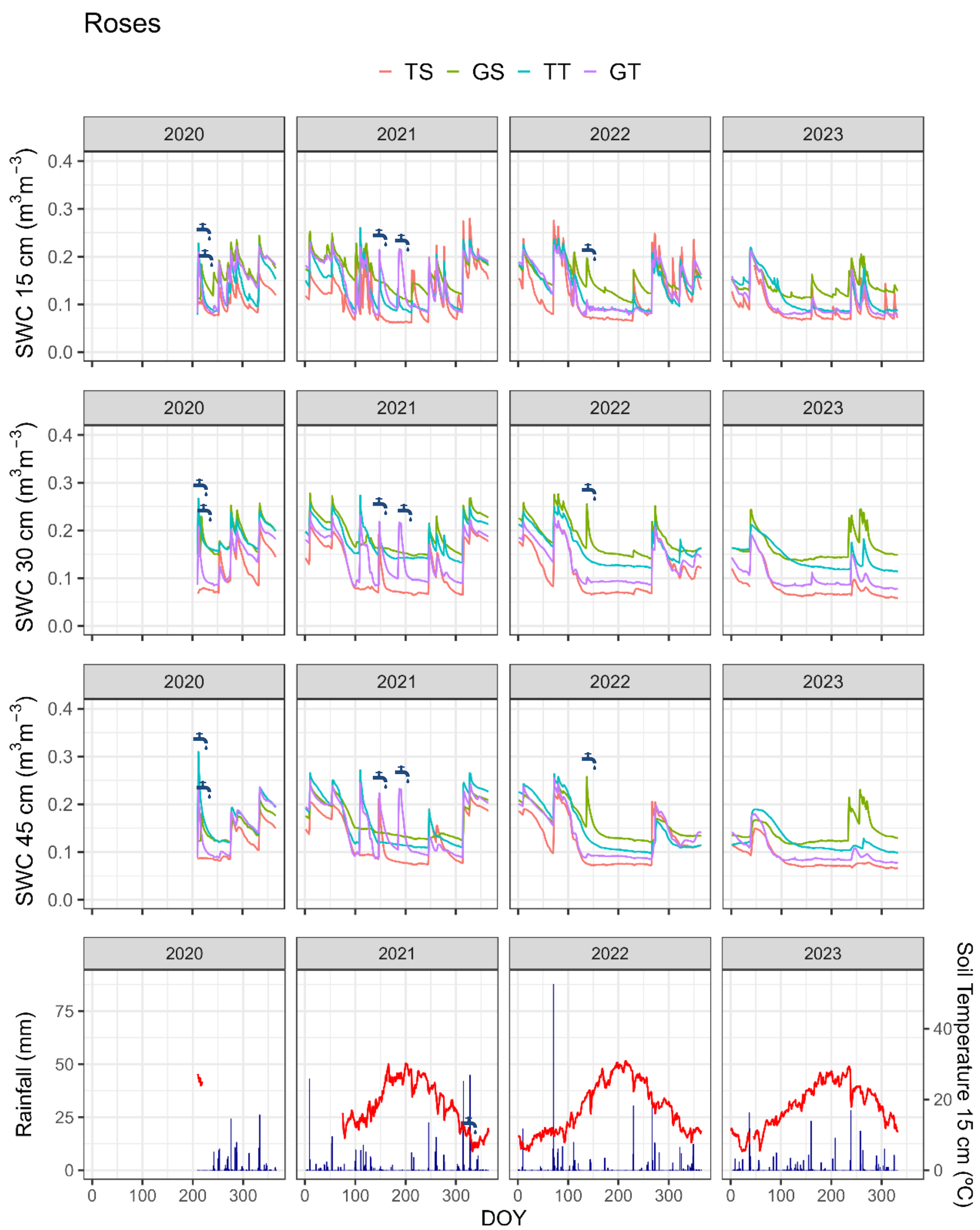


Figure 7. *Daily soil water content evolution in Roses pilot from August 2020 to December 2023. Each rectangle shows different sensor depth (15, 30 and 45 cm) and rainfall and soil temperature at 15 cm. Legend colour are the different agronomical practices. Tap symbols represent emergency irrigations or unplanned water leaks.*

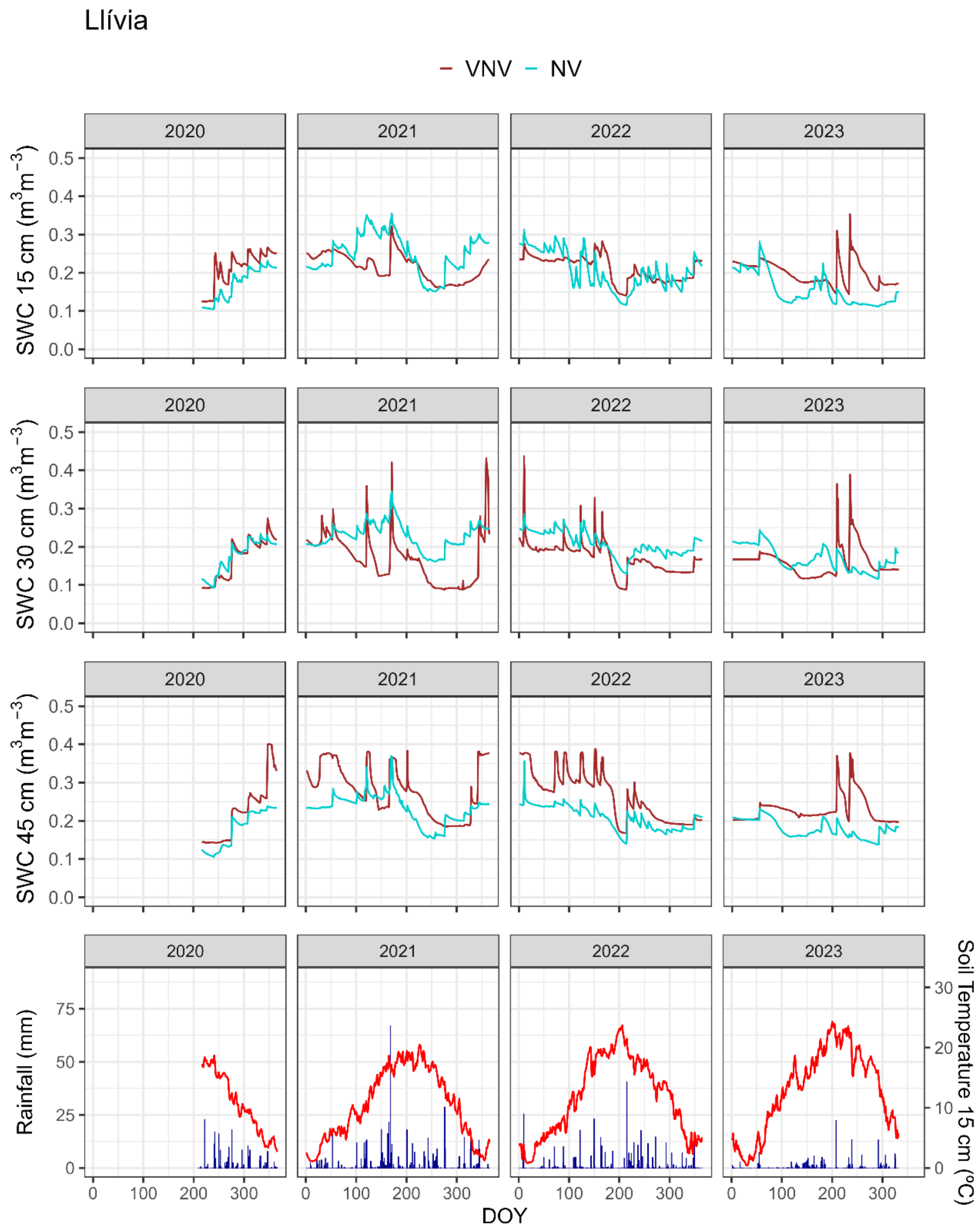


Figure 8. *Daily soil water content evolution in Llívia pilot from August 2020 to December 2023. Each rectangle shows different sensor depth (15, 30 and 45 cm) and rainfall and soil temperature at 15 cm. Legend colour are the different agronomical practices.*

For 2021, in Espolla pilot (Figure 6) we observed that NC presents an intermediate state between WE and CM (tilling + herbicides), responding similarly to CM in autumn (when the soil has not yet been recovering from summer drought) but quickly reaching similar SWC to WE, then following the same evolution till next spring, with CM presenting lower values along autumn and winter. In late winter (February-March) vegetation activation can be deduced from a decrease in SWC in all plots, although much slower in CM, which may be attributed to the absence of green cover. Sensibility to spring rains is again intermediate for NC, which joins SWC evolution of CM by the end of spring till next autumn, when the cycle repeats, which might be related to a still poorly developed green cover. It is expected that NC will tend to be similar to WE across seasons as its green cover will developed. In contrast, for 2022 WE and NC present throughout the whole year higher SWC values than CM, even in late winter. This could be because in 2022 rainfall filled the soil in all plots, while in 2021 it did not rain during late winter. During 2023, NC presented the lowest SWC and WE the highest, while the CM was intermediate. 2023 presented a dry vegetative season with high air temperature which increased the evaporative demand of soils. Also, results suggest the possibility of additional water depletion effect by the NC under these dry conditions. The highest values of SWC in WE during 2023, corroborate the positive effects of long-term soil cover for soil water retention.

In the Roses pilot, where combining vine conduction (Trellis vs Gobelet) and hillside management (Slope vs Terrace) (Figure 7), no clear pattern could be related with these conditions. The two plots presenting the lowest values shared no common factor: trellis + slope (TS) and Gobelet + terrace (GT). Some selective emergency irrigations or unplanned water leaks from the emergency irrigation system might have distorted the results (represented by tap symbols in Figure 7). However, both terraces (Trellis Terrace - TT and Gobelet Terrace - GT) seem to be more sensitive to spring rains as slope vineyards only respond near the soil surface. Gobelet + Slope (GS) is the least sensitive to spring and summer rains and keeps higher SWC most of these seasons. Interestingly, during 2023, GS and TT showed highest SWC at 30 and 45 cm, indicating a higher water availability to grapevine root system in these treatments.

In the Llívia pilot, precipitation is much more frequent and higher than in the other pilots, and the absence of a developed green cover and the small size of vines in the newest vineyard (VN, 1 year old vineyard) determine the evolution of SWC (Figure 7). Although deepest sensor is still sensitive to rain events all over the year, SWC is always highest at this depth, revealing small water capture by vines. In the new vineyard (N, 7 years old vineyard), higher canopy development, the presence of a spontaneous green cover and a straw mulching in the vine row (also present in VN, but not as consolidated as in N) result in a slower SWC dynamic, not so sensitive to rains but conserving more soil water in spring and most of summer, even with presumably a higher water extraction by vines. The 2023 year in Llivia was characterized by the lowest precipitations and highest temperatures. Under these condition VNV showed highest SWC at the different soil depths. It is important to note that a key viticulture management difference between VNV and NV exists; mulching is applied in NV but not in VNV. Considering the low response of soil sensors to precipitations during summer in NV, it is suggested that mulching had a negative effect in water infiltration in these climatic conditions (Figure 8).

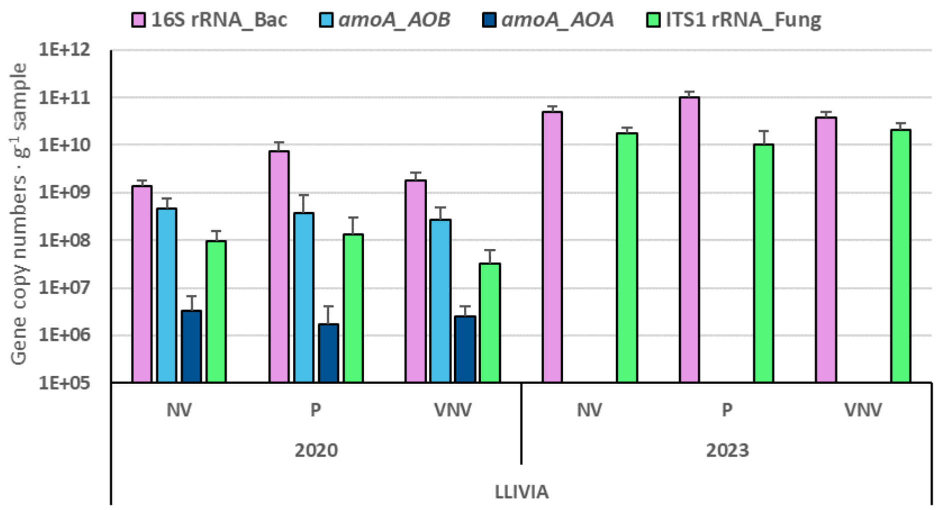
3.2. Monitoring results of microbial diversity

In 2023, a total of 68 samples (13 from Espolla veraison 2020, 25 from Roses, 15 from Llívia veraison 2020 and 15 from Llívia veraison 2023) have been analysed by qPCR (16S, ITS1 amoA (AOA) and amoA (AOB)). Regarding the microbial diversity assessment by 16S/ITS metataxonomy (paired end amplicon sequencing), only samples from Espolla (13) and Roses (25) are presented in this deliverable. The 30 samples from Llívia (veraison 2020 and 2023) will be sequenced and processed together in January 2024.

3.2.1. Microbial assessment at Espolla and Llívia sites

DNA extraction of soil material from each composite sample (5) for each crop management strategy, was performed by using DNeasy PowerSoil Pro kit (Qiagen), according to the manufacturer's instructions. Gene copy numbers of 16S rRNA (total bacteria), ITS1 rRNA (total fungi), ammonium oxidizing bacteria (*amoA* gene of AOB) and archaea (*amoA* gene of AOA) were quantified by quantitative real time PCR (qPCR) of each DNA extract (15 for conventional and 15 for ecological managed vineyards). The analyses were carried out using Brilliant II SYBR ®Green qPCR Master Mix (Agilent) in a Real Time PCR System MX3000-P (Stratagene, La Jolla, CA) as described elsewhere (Prenafeta-Boldú et al., 2012; Pelissari et al., 2018). For the standard curve of each target gene, it was designed five gBlocks® Gene Fragments (IDT, Integrated DNA Technologies). Ten-fold serial dilutions from synthetic genes were subjected to qPCR assays in duplicate showing a linear range between 10^1 and 10^8 gene copy numbers per reaction to generate standard curves. qPCR reactions fitted quality standards: efficiencies were between 90% and 110% and R^2 above 0.985. All results were processed by MxPro™ QPCR Software (Stratagene, La Jolla, CA) and were treated statistically. Results of total bacterial population (16S rRNA genes), ammonia oxidizing prokaryotes (*amoA*_AOB and *amoA*_AOA genes of ammonia oxidizing bacteria and archaea, respectively) and fungal population (ITS1 rRNA genes) are shown in Figure 8.

Both vineyards present a similar abundance of total bacterial populations (10^8-10^{10} 16S rRNA gene copy numbers/g sample), but in general, Espolla shows less total abundance (Figure 8). The highest microbial populations were observed in Llívia P management, being significantly higher in both years, 2020 and 2023, than the other treatments. The fungi population doesn't show significant differences among treatments. However, only in 2020, Espolla presents a higher ratio of fungi/bacteria population in all samples respect to Llívia (38%), even being not significantly different (Figure 9). Ammonia oxidizing prokaryotes (analysed only in 2020 samples) are present in all samples of both sites. Nonetheless, AOB populations are greater than AOA population. High nitrate levels are concomitant with the highest AOB/Bac ratio: Espolla NC and Llívia NV. This fact could be explained due to the activity of nitrite oxidizing bacteria (NOB) which depends on simultaneous AOB activity. Interestingly, a high increase of the ratio of fungi/bacteria was observed in 2023 in Llivia, reaching ratios of 32% in NV (9% in 2020) and 65% in VNV (3% in 2020), even above than observed in well stablished covers in Espolla in 2023 (34% in 20201 and 41% in 2020). In addiitona a higher bacterial and fungal biomass was depicted in Llivia 2023 (values above 10^{10} gene copies · g^{-1} both in bacterial and fungal populations) than in Espolla (values in the range of 10^9 gene copies · g^{-1} for bacteria and 10^8 gene copies ·g^{-1} for fungal populations) a)



b)

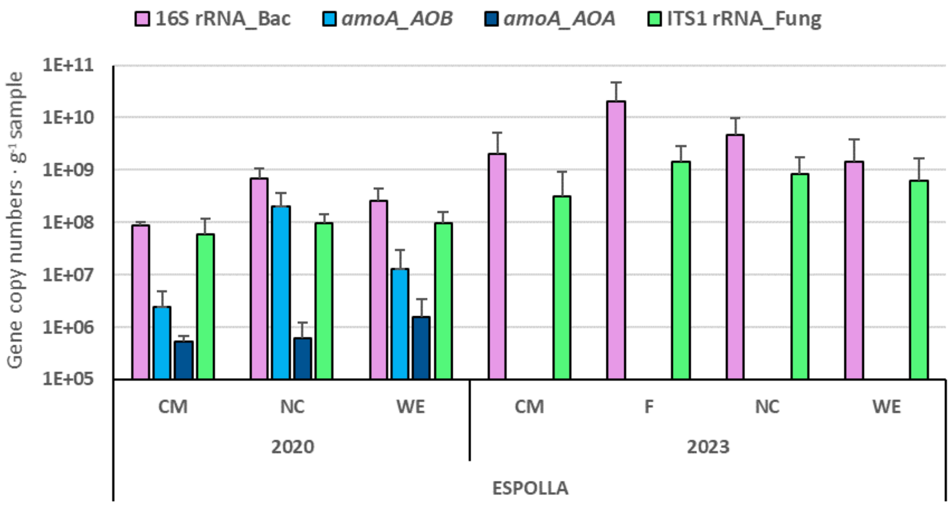


Figure 8. *Abundance of microbial biomass quantified by qPCR in Llívia (a) and Espolla (b) (veraison 2020 and 2023 sampling campaign) of different agricultural practices. Total bacterial population (16S rRNA); AOB (ammonia oxidizing bacteria); AOA (ammonia oxidizing archaea); total fungal population (ITS1 rRNA). Presented values are the mean and SD of independent replicates (n=5). Espolla: WE: Well Established Cover, NC: New Cover; CM: Conventional management. Llívia: New vineyard refers to recently establish vineyard (NV); Very new vineyard refers to a very recently established vineyard (VNV): Pasture refers to nearby pasture (P). The results regarding AOA and AOB in 2023, and the ratio of Fungi/Bacteria are pending to be processed and will be included in 2024.*

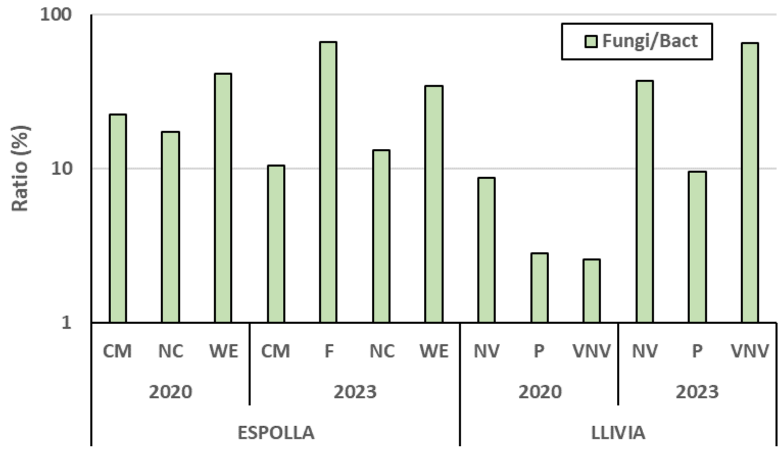


Figure 9. *Bar charts represent the ratio of fungal population versus total bacterial population (16S rRNA). Espolla: WE: Well Established, NC: New Cover; CM: Conventional management. Llívia: New vineyard refers to recently establish vineyard (NV); Very new vineyard refers to a very recently established vineyard (VNV); Pasture refers to nearby pasture (P).*

3.2.1.1. Optimized protocol for microbial community assessment

An optimized protocol for analysing microbial community assessment by paired end amplicon sequencing of 16S (total Bacteria) and ITS2 libraries (Total Fungi) have been implemented as follows (for all sites of the project).

To determine the bacterial communities of soil samples, 16S rRNA gene libraries were sequenced by paired-end High Throughput Sequencing (HTS). DNAs were sequenced by utilizing MiSeq (Illumina, 2×300 bp kit), following the manufacturer's guidelines, at Molecular Research DNA facilities (USA, Texas). Total DNAs were amplified by using the bacterial V3-V4 hypervariable region in the 16S rRNA libraries, the primers were V3_341F (5'-CCTACGGGNGGCWGCAG-3′) and V4_R805 (5'-GACTACHVGGGTATCTAATCC-3′).

Raw data (R1 and R2 demultiplexed FASTQ files) from 16S rRNA (bacteria), were further processed using Cutadapt and DADA2 software (Callahan et al., 2016). Forward and reverse primers were removed from the raw paired ended reads by means of the Cutadapt (Martin, 2011) in QIIME2 Software (version 2021.11). Then, the resulting paired reads were exported to RStudio (version 4.1.2), and filtered and trimmed, denoised and merged using the R package DADA2 (Callahan et al., 2016). R1 and R2 reads were truncated (*truncLen*) to 260 and 240 for 16S rRNA. In all samples, reads with ambiguities or an expected error (*maxEE*) higher than 2 were discarded. The DADA2 denoising algorithm was applied to determine an error rates model to infer true sequence variants (ASVs). The full denoised amplicon sequence variants (ASVs) were obtained after merging the denoised R1 and R2 sequences using a minimum overlap of 12 bp. Finally, chimeras were detected and re-moved using the function *removeChimeraDenovo*() as described elsewhere in the DADA2 1.16 tutorial (https://benjjneb.github.io/dada2/tutorial.html). The taxonomic affiliations of the ASVs for total bacteria were assigned by using the naïve Bayesian classifier method (Wang et al., 2007), using the RDP database for 16S rRNA (bacteria), and compiled into each taxonomic level (DeSantis et al. 2006). For assignment, the RDP Bayesian Classifier was set with a bootstrap cut-off of 80%.

To assess alpha diversity, Shannon (H), Inverted Simpson (I/D), Richness (Sobs) and Chao 1, indexes, based on rarefied samples (6393 reads), were calculated by using the Mothur software (version 1.46.1). MicrobiomeAnalyst (Chong et al., 2020) and Phyloseq R Package were used to visualize and assess the distribution of the main taxonomic groups in relative abundance based on rarefied samples. For beta diversity assessment, the differences in overall community composition between samples were calculated using the Bray-Curtis dissimilarity distance in rarefied and normalized samples by total sum scaling ASVs as recommended by McKnight et al. (2019), and ordinated 2D by means PCoA analysis. The contribution of each management crop practice to the microbial community dissimilarity were assessed by means of non-parametric permutational multivariate analyses of variance (PERMANOVA) and by analysis of similarity (ANOSIM) of total ASVs rarefied distributions, based on Bray-Curtis distances with 999 permutations, was conducted in Vegan R package by means *adonis2()* and *anosim()* functions respectively.

Differential abundance analysis of representative sequence variants (ASVs) was performed by conducting basic univariate tests for two-group comparison, Wilcoxon tests, and adjusted p-values by False Discovery Rate (FDR), by using rarefied abundance ASV matrix, and considering those ASVs with more than 5 reads in at least 20% of samples, by using MicrobiomeAnalyst and Microbiome R Package.

3.2.1.2. Microbial diversity assessment in Llívia soil samples

Regarding Llívia site, the results regarding the soil microbial diversity assessment conducted by means of paired end amplicon sequencing (16S/ITS-Miseq) are not shown in this deliverable. The results at Llívia will encompass 2 sampling campaigns (veraison 2020 vs veraison 2023). The processed diversity results are expected to be available at the beginning of 2024, as the soil samples of both sampling campaigns will be sent all together to be sequenced by paired end amplicon sequencing at the end of 2023. By this way, the diversity assessment of 16S/ITS2-metabarcding will be more precise, comparing samples from 2020 and 2023 from the same site, together analysed in the same run of MiSeq sequencing).

3.2.1.3. Microbial diversity assessment in Espolla soil samples (veraison 2020)

Most predominant fila depicted in Espolla site were Actinobacteria (25% in C (WS) and 30% in NC), Acidobacteria (28% in C (WS) and 20-25% in NC) , followed by Proteobacteria (20-25% in C (WS) and 25% in NC), Firmicutes (6-8% C (WS) higher than 3-4% in NC) , Verrucomicrobia (3-6% in C (WS) and 6% in NC) , Chloroflexi (5-6% in C (WS) and NC), Gemmatimonadetes (1-3% in C (WS) and at 1% in NC) and Nitrospirae (1% both in C (WS) and NC). New young cover depicted higher relative abundance of Actinobacteria, Proteobacteria and Verrucomicrobia, while Acidobateria and Firmicutes were more enriched in well stablished cover.

Figure 10. *Microbial community taxa distribution in vineyard soils in Espolla (veraison 2020) (-5/-20cm) at Phylum level (Top) and genera level (Below). WE: Well Established cover (C) and NC: New Cover (NC) are presented; CM (conventional management) failed the analysis and will be repeated in 2023. Classes and Phyla >1% of relative abundance are reported.*

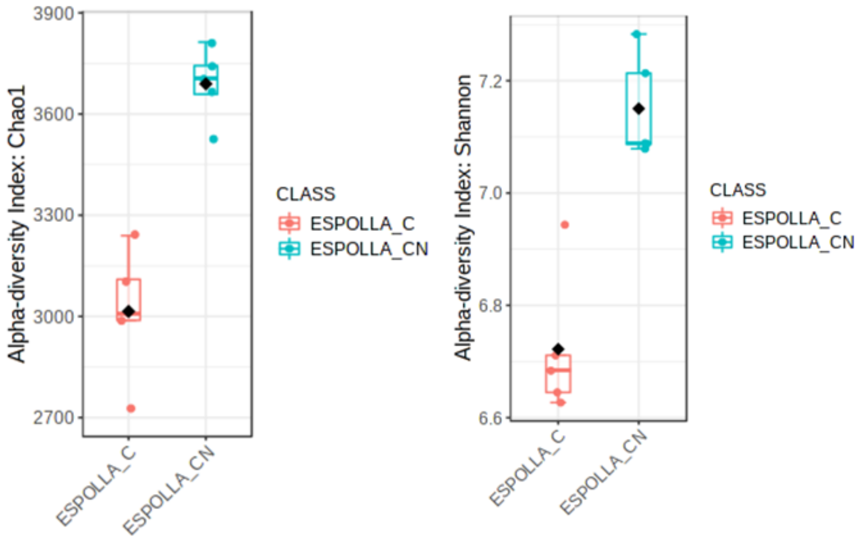
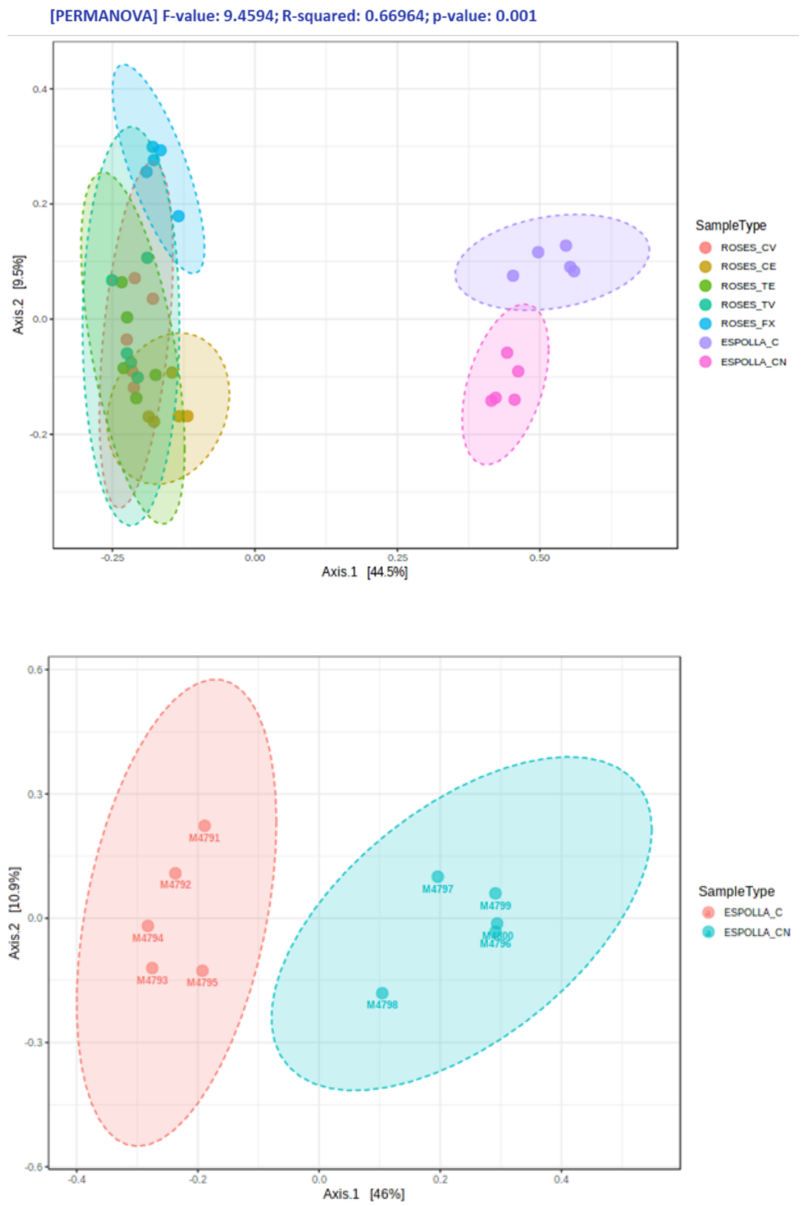


Figure 11. *Boxplots of Alpha diversity indexes (n=5/treatment), of richness (Chao1) and diversity (Shannon) in Espolla site (veraison 2020).*

Regarding alpha diversity in Espolla, it is noteworthy that the soil with well stablished cover (C (WS)) has a significant lower Richness (Chao 3000) and diversity (Shannon index of 6,65) than soil with young cover (NC) (Chao; 3700, and Shannon of 7,15) (P: 0.0003, and P: 0.0079, Mann-Whitney). Such differences in alpha diversity were also confirmed regarding beta diversity. Beta diversity results are shown in Figure 11 in 2D diagrams of PCoA, based on Bray Curtis dissimilarity values obtained from Espolla and Roses site. The PCoA and Permanova analysis revealed an effect ($R2$: 0.6696 $p<0.001$) of the age of the cover to differentiate the microbial diversity (at ASV level distribution) in Espolla site. In addition, the diversity of soil in Espolla were clearly different in beta diversity than in Roses site.



[PERMANOVA] F-value: 6.2048; R-squared: 0.43681; p-value < 0.01
[ANOSIM] R: 1; p-value < 0.01

Figure 12. *PCoA 2D ordination (Bray Curtis distance) revealing the effect of different crop management in the dissimilarity of microbial diversity structure in the vineyard soil (-5 to -20cm) at Espolla vs Roses (Top) and Espolla C vs CN (bottom). Permanova analysis revealed a significant effect of crop management effect on Bacterial diversity at Espolla (F-value 6,20; R2: 0,437; p-value <0.01) and Roses site between sites: [PERMANOVA] F-value: 9.4594; R-squared: 0.66964; p-value: 0.001.*

3.2.2. Microbial assessment of soil samples from Roses site (veraison 2021)

The quantitative assessment of the microbial abundance in soil, quantify by gene abundance by qPCR is shorn in the Figure 13.

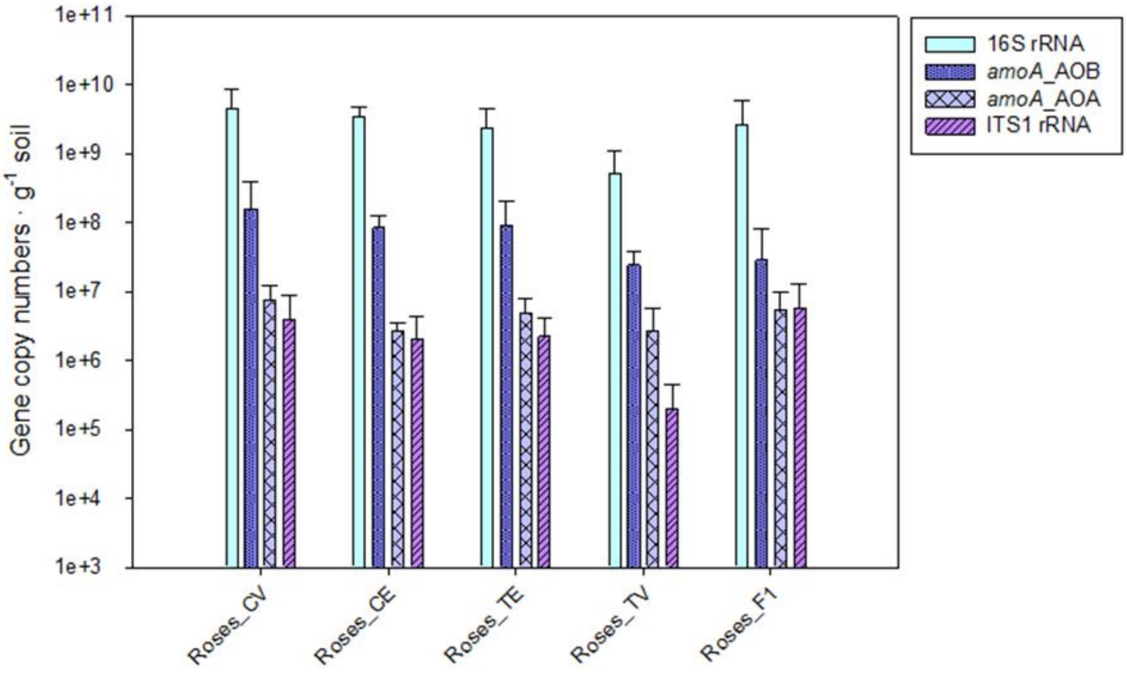


Figure 13. *Abundance of microbial biomass quantified by gene populations by qPCR in Roses (veraison sampling 2021). The Roses plots are: CE: Trellis slope (TS), TE: Trellis Terrace (TT), TV: Gobelet Terrace (GT); CV: Gobelet Slope (GS); and F1: Scrubland (S). Total bacterial population (16S rRNA); AOB (ammonia oxidizing bacteria); AOA (ammonia oxidizing archaea); total fungal population (ITS1 rRNA). Presented values are the mean and SD of independent replicates (n=5)*.

Total bacterial populations achieved a high range of population about 10^9 16SrRNA copies/g soil in all treatments except for TV (GT) with a lower total bacterial population (10^8 16S copies/g). Regarding ammonium oxidizing bacteria, such population was in slope plots and Trellis Terrace (TE) with a population close to 10^8 *amoA*/g, being lower again (P<0.05) in TV (TG) and control scrubland, achieving 10^7 copies *amo*A/g. Regarding total fungal populations the range were mainly in 10^6 ITS1/g except again in TV (GT) with the lowest population (10^5 ITS1/g) (P<0,05). Scrubland plot showed a slightly (but not significant) AOA and fungal population than vineyards.

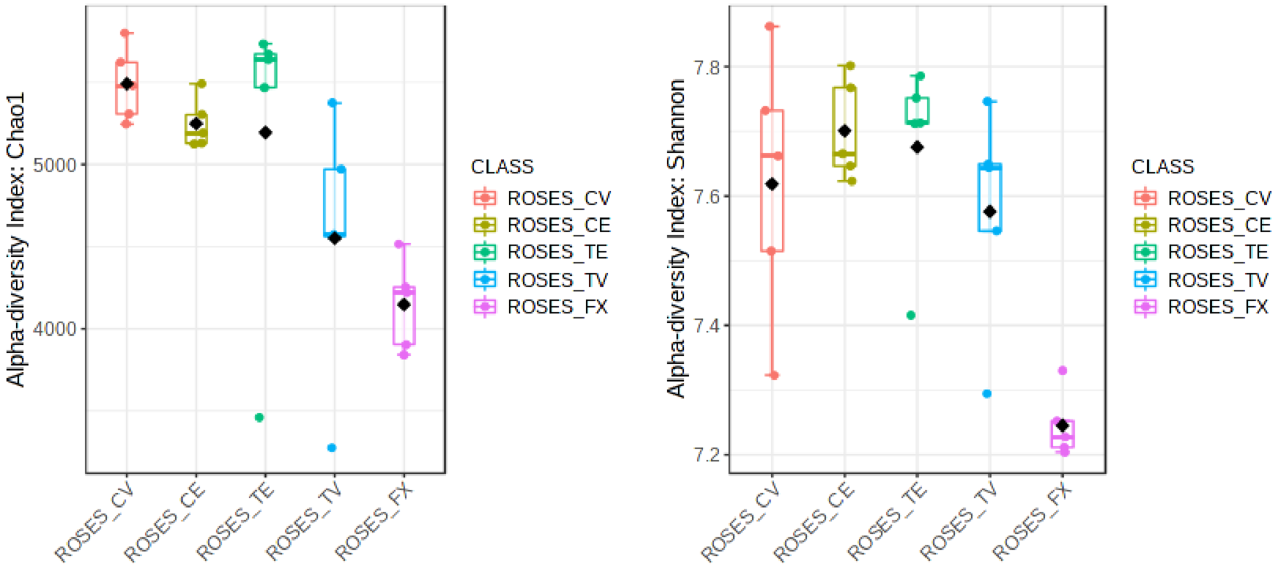
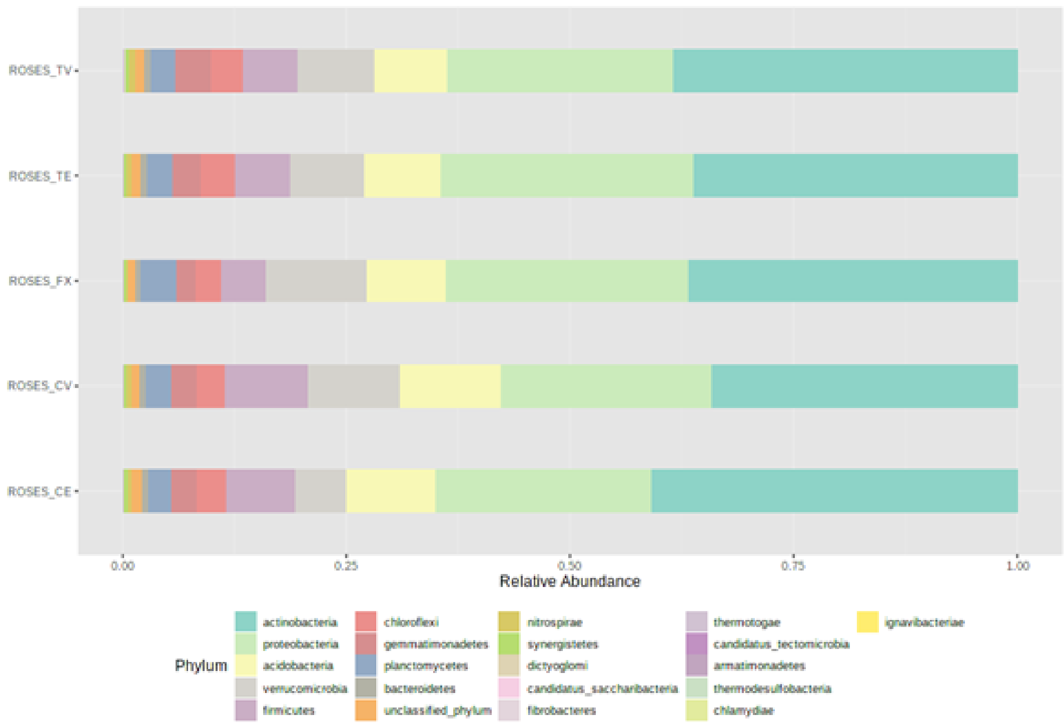


Figure 14. *Boxplots of Alpha diversity indexes (n=5/treatment), of richness (Chao1) and diversity (Shannon) in Roses site (veraison 2021). The Roses plots are: CE: Trellis slope (TS), TE: Trellis Terrace (TT), TV: Gobelet Terrace (GT); CV: Gobelet Slope (GS); and FX: Scrubland (S).*



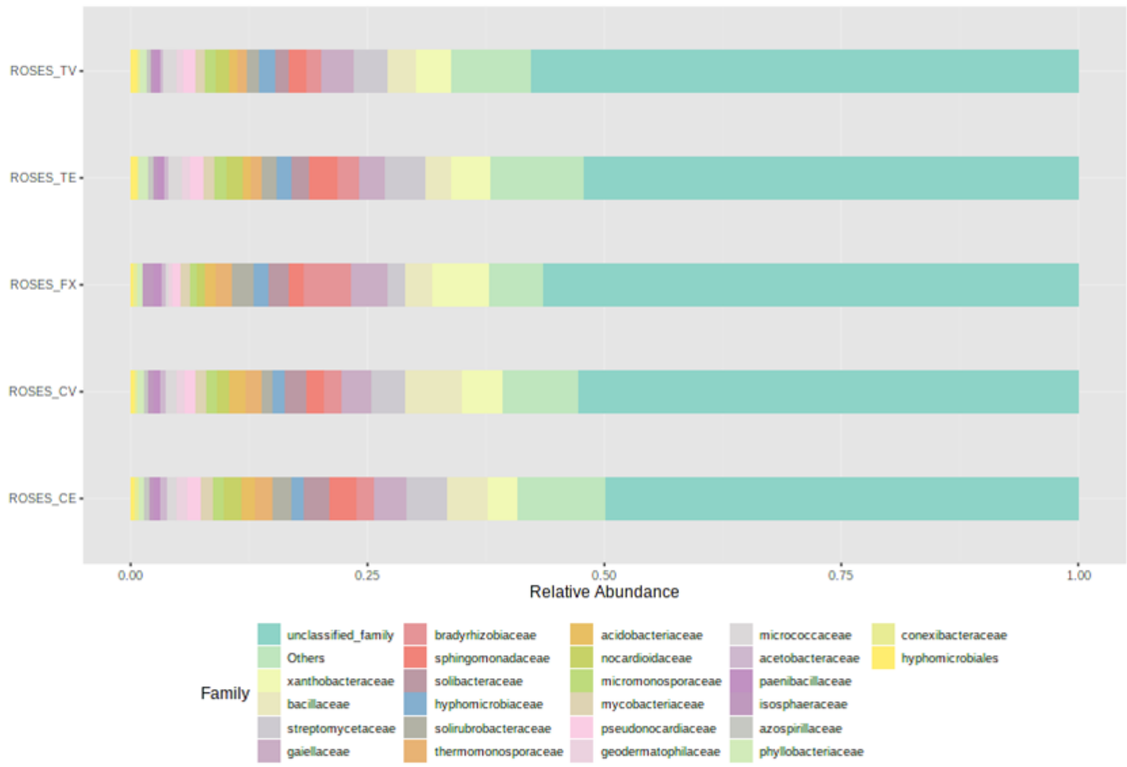


Figure 15. *Microbial community taxa distribution in vineyard soils in Roses (veraison 2021) (-5/-20cm) at Phylum (TOP) and family level (bottom). Phyla and families >1% of relative abundance are reported. The Roses plots are CE: Trellis slope (TS), TE: Trellis Terrace (TT), TV: Gobelet Terrace (GT); CV: Gobelet Slope (GS); and F1: Scrubland (S). Presented values are the mean and SD of independent replicates (n=5).*

Regarding alpha diversity results in soil samples from Roses site it's in noteworthy that the presence of vine was linked to a higher richness and diversity (P>0.005) (Chao1 and Shannon index of 4700-5500 and 7,6-7,7 in vineyards vs Chao of 4100 and Shannon 7,2 in Scrubland. The results revealed the positive effect of vine plant to boost microbial diversity and richness in soil in Roses site. In addition, again TV (GT) showed a lower richness than the rest of treatments in vineyards. No significant differences were observed regarding the Shannon index in the different treatment in the vineyards in the presence of vine plant.

Regarding microbial community composition, main predominant Fila in Roses were (from top to bottom): Actinobacteria (37,4±2,5%) and Proteobacteria (25,5±2,0%) (class α), Acidobacteria (9,5±1,3%, clearly lower than in Espolla (25-30%), Firmicutes (6,9±1,7%), Verrucomicrobia (8,7±2.1%), Chloroflexi (3,5±0,3%) and Gemmatimonadetes (3,0±0,6).

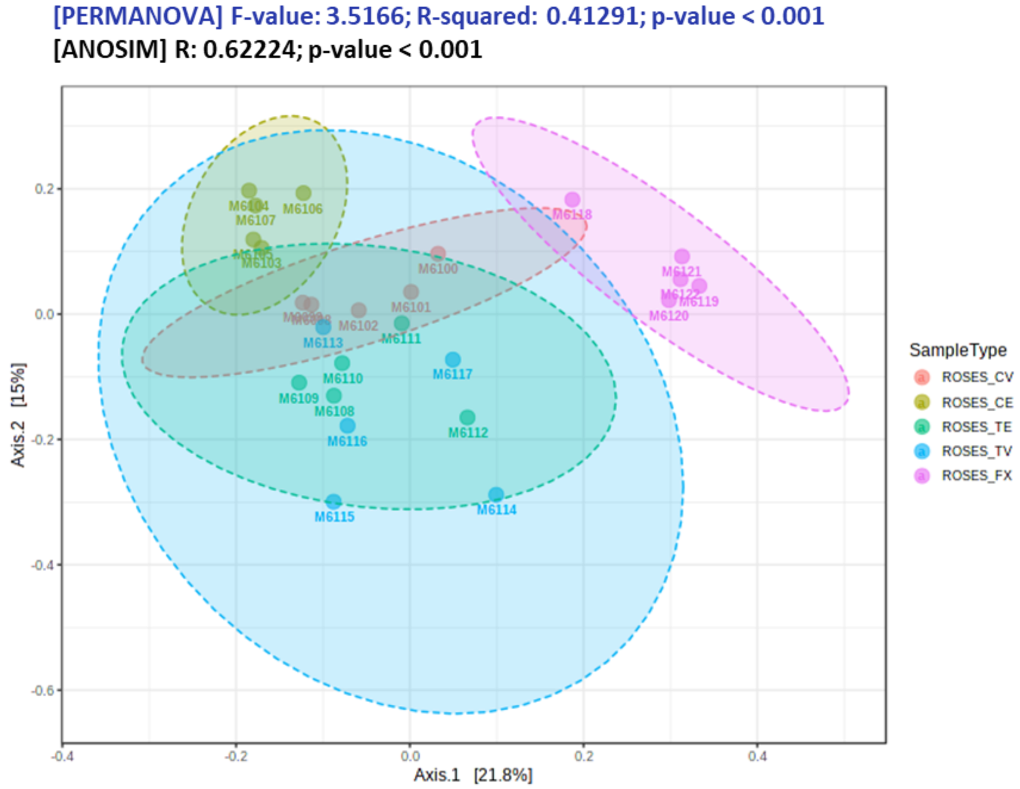


Figure 16. *PCoA 2D ordination (Bray Curtis distance) revealing the effect of different crop management in the dissimilarity of microbial diversity structure in the vineyard soil at Roses site (-5 to -20cm). Permanova assessment revealed a significant effect of crop management effect on Bacterial diversity in Roses site: [PERMANOVA] F-value: 9.4594; R-squared: 0.66964; p-value: 0.001.*

Regarding beta diversity, PCoA of Bray Curtis distance of bacterial dissimilarity, and Permanova assessment revealed a significant difference in diversity (ASVs level) due to vine plant presence and in slope plots compared with terraced plots (Roses F: 5,318 p<0,001).

3.3. Monitoring results of vineyard production

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.

We have received grape production per hectare and alcoholic by volume (ABV) data since 2011 to 2023 from Espolla; since 2017 to 2022 in Llívia (the first year with productivity in their vineyards); and in the Roses case, they have only provided us 2022 data. We are expecting to receive 2023 data from Llívia and Roses.

In Espolla, yield of CM is higher than NC and WE (Figure 17a). Regarding alcoholic strength, in general the lower values are showed by CM and the highest by WE, with NC presenting intermediate values. In Roses, there are no significant differences in production between the different practices. Although, the production in trellis plantation is slightly higher (around 800 kg/ha) than in gobelet (around 520-650 kg/ha), as expected. Alcoholic (ABV) strength present the reverse pattern, with higher values in gobelet pilots than terrace pilots. In Llívia, yield is improving with time as expected on a young vineyard (Figure 17b). In conclusion, the productivity is now higher in conventional management, but we could expect an increase in yield over time for green cover pilots. Similarly, less productivity generally implies a higher alcoholic strength.

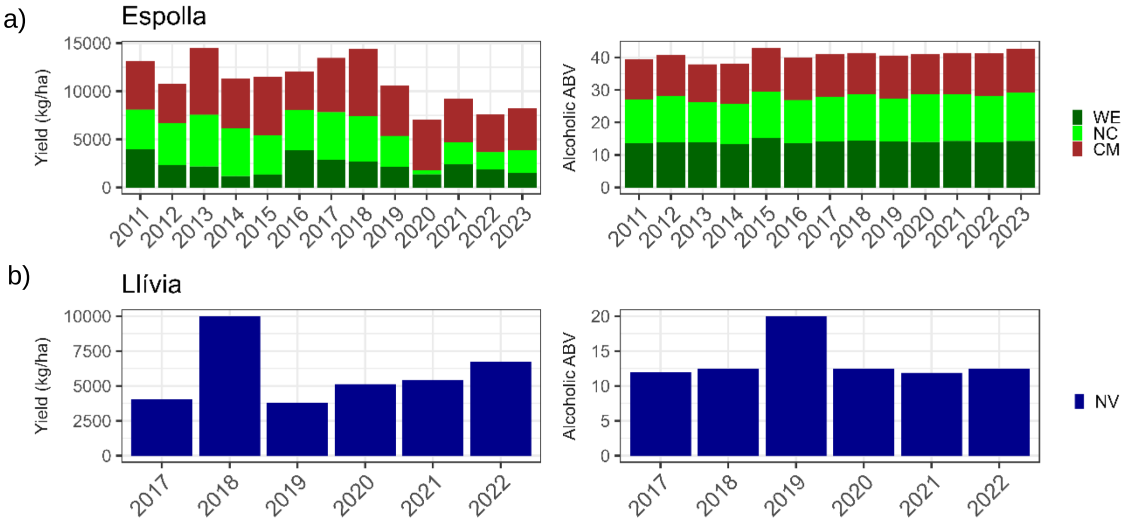


Figure 17. *Annual yield and alcoholic ABV for Espolla (a) and Llívia (b). Legend colour are the different agronomical practices.*

3.4. Monitoring results of 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 for a long time, affects the vegetation cover, which in turn affects interception and evapotranspiration of the plants, and 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 have been carrying out rainfall simulation experiments (Iserloh et al., 2012) seasonally in all monitoring plots and control plots (non-vineyard sites. Fennel in Espolla and Pasture in Llívia) in Catalonia. The first campaign in October 2020 (considered as wet soil conditions), the second in July 2021 (in very dry soil conditions) and the third in October 2022 (repetition of the wet conditions). The soil water content in the 2021 dry conditions for all pilots in Empordà (Roses and Espolla) is around 1% and in Llívia is around 9.6%. In general, in October 2020 the soil conditions are less wet (around 11%) than in October 2022 (around 13.5%), except for Llívia pilot (around 33% in 2020 and 18% in 2022) (data not shown).

Table 3*. Mean hydrogeological and sedimentological variables extracted from rainfall simulations in Espolla, Llívia and Roses in 2020, 2021 and 2022 campaigns. RI: Rainfall Intensity (mm h^{-1}), RC: Runoff Coefficient (mm mm^{-1}), WF: Wetting Front (cm), SC: Sediment Concentration (g L^{-1}), ER: Erosion Rate (g m^{-2}). WE: long-established cover crop, NC: Newly established cover crop, CM: Conventional soil management, F: fennel, NV: recently establish vineyard, VNV: very recently established vineyard, P: pasture, TT: Trellis Terrace, TS: Trellis Slope, GT: Gobelet Terrace, GS: Gobelet Slope and S: Scrubland.*

Site	Agricultural practices	Year	Slope (%)	RI (mm h^{-1})	RC (mm mm^{-1})	WF (cm)	SC (g L^{-1})	ER (g m^{-2})
Espolla	WE	2020	14.05	46.50	0.03	8.33	1.98	0.05
		2021	14.05	89.50	0.10	8.67	1.67	0.55
		2022	14.05	59.33	0.01	19.67	1.76	0.04
	NC	2020	0.00	49.05	0.04	11.00	1.27	0.14
		2021	0.00	43.50	0.06	9.33	5.85	0.47
		2022	0.00	67.25	0.11	15.50	2.11	0.47
	CM	2020	1.75	61.25	0.32	9.67	0.62	0.40
		2021	1.75	33.25	0.33	6.33	12.99	0.55
		2022	1.75	60.50	0.30	7.83	4.01	2.89
	F	2020	0.00	57.75	0.01	7.00	2.44	0.09
		2021	0.00	53.50	0.00	7.67	0.00	0.03
		2022	0.00	62.00	0.00	19.67	43.60	0.23
Llívia	NV	2020	6.99	65.00	0.01	9.00	5.22	0.23
		2021	6.99	106.00	0.20	16.67	2.11	1.88
		2022	6.99	65.33	0.01	9.67	9.86	0.35
	VNV	2020	7.87	58.25	0.14	10.33	5.20	2.51
		2021	7.87	99.00	0.26	17.67	1.26	1.55
		2022	7.87	56.00	0.03	6.67	6.81	0.44
	P	2020	11.39	89.25	0.73	NA	0.30	0.74
		2021	11.39	81.00	0.46	5.67	0.29	0.82
		2022	11.39	63.00	0.19	11.00	0.87	0.58
Roses	TT	2021	7.57	85.50	0.34	18.00	0.56	1.34
		2022	7.57	69.00	0.01	24.00	2.44	0.07
		2020	0.00	45.67	0.01	8.00	3.94	0.23
	TS	2021	3.49	98.50	0.14	16.00	2.12	0.75
		2022	3.49	64.50	0.00	25.67	4.82	0.07
		2020	7.57	41.50	0.06	12.17	1.98	0.19
	GT	2020	8.17	29.00	0.03	10.33	12.33	1.08
		2021	9.63	65.00	0.01	31.00	0.00	0.25
		2022	9.63	73.00	0.01	13.00	6.69	0.08
	GS	2020	13.75	41.75	0.03	10.67	4.84	0.24
		2021	12.28	60.50	0.01	NA	3.63	0.11
		2022	12.28	71.00	0.01	25.00	5.34	0.25
	S	2021	0.00	101.00	0.07	14.00	1.46	0.56
		2022	0.00	77.50	0.02	7.33	0.77	0.18

All rainfall simulations last 20 minutes and if they last longer, the results have been corrected so that they can be compared with each other. Nevertheless, we will consider improving this correction using other methodological approaches such as pedrotransfer functions, with the aim of estimating the increase of soil water content for the simulations longer than 20 minutes.

In each experiment, several variables characterizing the hydrogeological and sedimentological response are obtained: Infiltration rate (mm h^{-1}), Infiltration coefficient (mm mm^{-1}), Runoff coefficient (mm mm^{-1}), Erosion rate (g m^{-2}), Sediment concentration (g l^{-1}), Time to runoff (min), Wetting front (cm), Sediment production (g) and Sediment detachment (g m^{-2} h^{-1}), among others (Table 3 and Figure 18).

All statistical analyses were carried out with R Statistical Software version 4.1.1 (R Development Core Team, 2021). We used mixed-effects models (package nlme, Pinheiro & Bates, R Core Team, 2021), specifying reply as a random factor, to analyse management differences on hydrogeological and sedimentological variables. We graphically inspected residual plots and implemented appropriate variance structures to minimise heteroscedasticity in the residuals (varPower structure in nlme/lme functions). This generic function fits a linear mixed-effects model by restricted maximum likelihood (REML). Package emmeans (Russell, 2021) was used to estimate marginal means (EMMs, least-squares means) for factor combinations of the model. Compact letters display (CLD) was obtained with cld function of pairwise comparations, which extract and display information on all pairwise comparations of least-squares means (Piepho, 2004). For all statistical tests, significance was accepted at $p < 0.05$. Figures were produced using the R package ggplot2 (Wickham, 2016).

In Espolla CM plot, the runoff coefficient is higher than the NC and WE plots (Figure 18). In parallel, the infiltration coefficient in the 2021 dry campaign shows higher values for CM and lower values for WE. This pattern is reversed in 2020 and 2022 wet campaigns. If the soil is wet, there are no significative differences in the IC among management practices. On the other hand, if the soil is dry (as happened in 2021), IC is higher in CM than WE, NC, and F. Another dry campaign would be needed to confirm this pattern.

On some occasions, CM presents superficial crust which reduce water infiltration. In the WE cover plot, we find lower runoff values than the NC plot, due to the interception effect of water on well-established vegetation. Vegetation cause water retention on the surface for longer and water has a better chance of infiltrating. In case of fennel (F), there is no runoff as the soil is totally covered and protected by vegetation. But the infiltration rate is not the highest, so the difference must be in water intercepted by vegetation.

The sediment concentration in the runoff water does not vary too much between plots, although the variability is greater in the WE and the F, and this is reflected in the erosion rate. There seems to be a simulation in each case with much more sediment dragged per unit volume for a similar runoff, which results in this pair of simulations giving higher runoff rates. We find that, in correspondence with what happens with runoff, the erosion rate is higher in the CM plot, in the 2022 campaign above all. Also, the NC has higher erosion values than the WE, which has similar values than F, but without statistical differences.

In Roses, there are no statistical differences in the rate of erosion. Differences in sediment concentration play a remarkably key role, which could point to a singular difference in soil surface (different texture from the other plots or different cover condition or sloping direction...). However, there are no significant differences between vineyards with different forms of planting. Regarding runoff, it is slightly larger in the TS than the TT, but again there are not statistical differences. In case of gobelet vineyards, runoff is slightly higher in terrace than slope. There is apparently no clear pattern in runoff in the case of the distribution of the grapevine in gobelet or trellis. Both plots with higher runoff (TS and GT), coincide in being the two with the lowest soil water content in the soil throughout the year (Figure 6), although this is not seen in the infiltration, and runoff levels are very low.

The pattern in the infiltration rate is the same as with the erosion coefficient, which is not at all expected. It should be noted, however, that the runoff coefficient is always very low, which leads to the question of whether the differences in infiltration are not only due to differences in precipitation between the four plots due to the wind. In fact, following the same pattern the runoff coefficient and the infiltration rate have already point out that an important component of the differences between treatments is the difference in precipitation. If we represent the infiltration as a proportion of the rain, which eliminates this component, we can see that, except for TT, the infiltration coefficient and the runoff coefficient does follow a symmetrical pattern, when one goes up the other goes down. An exception would be, as we have said, the TT. It has the lowest infiltration coefficient and the lowest runoff coefficient, making us wonder where the remaining water has gone (the two of them only reach about 60% of the rain). The cover retention would be ruled out by low coverage (only one of the three simulations reaches 60%, the other two being below 10%, at the same level as other treatments. It was measured on a different day from the rest, with wind, which would result in much less water reaching the circle, although in the rain gauges the records are the highest in Roses (which, if true that part of this water does not reach the ground, would contribute to lower the proportion of both runoff and infiltration. The other possible component is evaporation, stimulated by the wind, which would justify the difference between infiltration + runoff on one side and precipitation on the other. Higher wind values would increase evaporation. Infiltration rate is higher during the dry 2021 campaign for TS and S.

In Llívia, the runoff was between 12 and 27 mm in the P plot and, on the other hand, the VNV, with a bare soil, the rates are up to 4 mm. In the VNV, there was a straw cover, manually added, and almost no vegetation. In the NV, the runoff was very low or practically non-existent. The cover in this vineyard is established based on low cover and straw. Erosion rate is the result of the sediment transported per unit volume and the runoff produced. There may be a high runoff, but if it does not transport sediment, the total erosion will be low, as happens in the Llívia pasture.

There is a high range in the erosion rate in the VNV (between 0 and 45 g/m^2 approximately), while the erosion rates in the NV are very low. In the case of P, the erosion rate is very stable between measurements, around 5 g/m^2. If we look at the data from the point of view of sediment concentration, the P cover seems to protect very well from erosion, and remarkably high runoff resulted in minimal erosion that can exceed the NV one, which it has a lower erosion rate. But, due to the much lower runoff, and not to the concentration of sediment, which is higher than in the P and similar with the VNV. The infiltration rate in Llívia is symmetrical with the runoff coefficient, as well as happen in Espolla, except for the F already explained before.

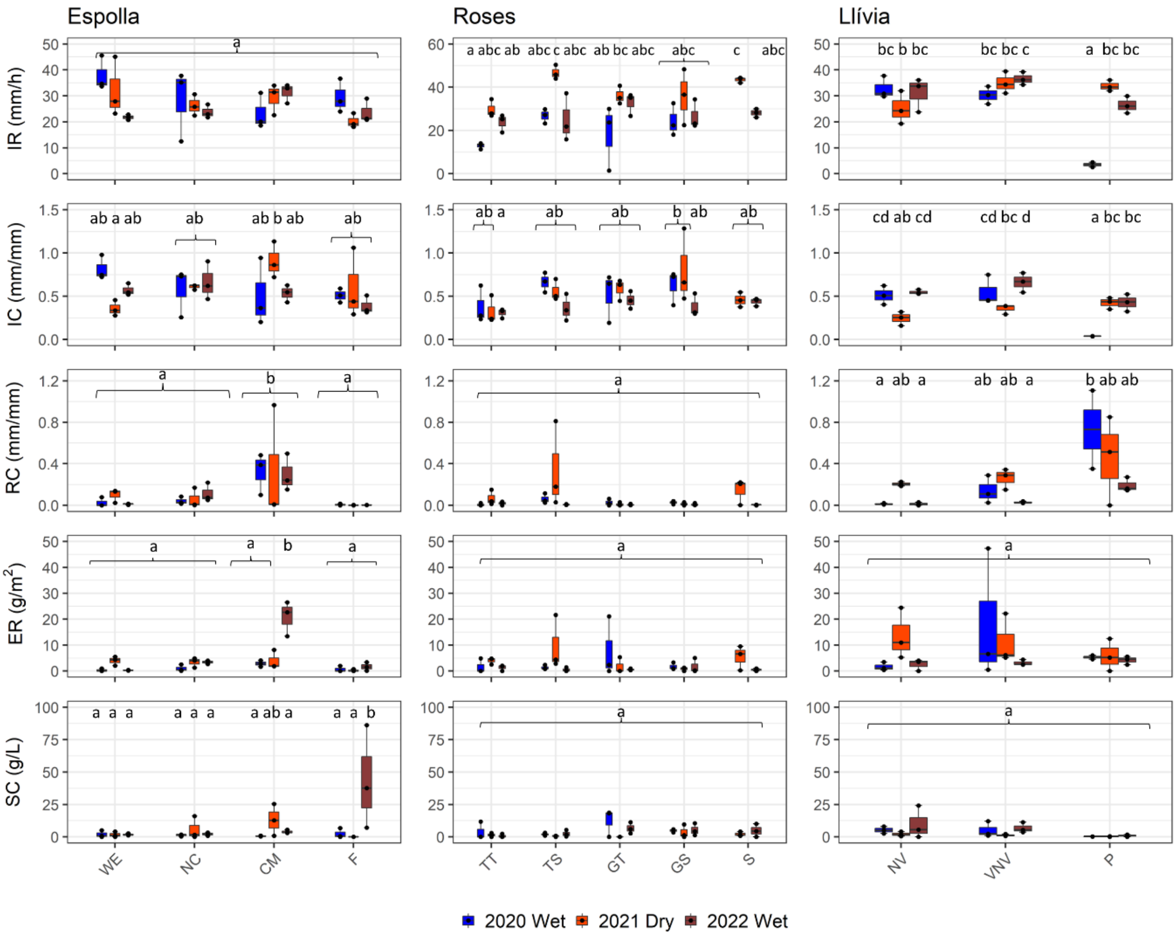


***Figure 18**. Box plot of hydrogeological and sedimentological variables extracted from rainfall simulations in Espolla, Roses and Llívia plots in October 2020, July 2021, and October 2022. IR: Infiltration rate (mm h^{-1}), IC: Infiltration coefficient (mm mm^{-1}), RC: Runoff coefficient (mm mm^{-1}), ER: Erosion rate (g m^{-2}) and SC: Sediment concentration (g l^{-1}). Blue boxes represent 2020 (soil wet conditions), red boxes represent 2021 (soil dry conditions) and brown boxes represent 2022 (soil wet conditions). The box portion of the box plot is defined by two lines at the 25th percentile and 75th percentile. The two whisker boundaries are the 5th and 95th percentile. Lowercase letters indicate significant differences between plots and years (p<0.05).*

3.5. Monitoring of site meteorological conditions

The registration of the meteorological conditions is key to understand the evolution of previous variables along the project duration. In Catalonia plots, all three sites have nearby meteorological stations of the SMC (Servei Meteorològic de Catalunya) or ICGC in case of Llívia plot (since June 2021), sometimes adjacent to the plots (Table 4). This publicly available information has been used to represent site meteorological conditions, included rainfall in soil moisture figures (Figure 5, 6 & 7).

Table 4. *Reference meteorological station per experimental plots.*

Site	Reference Meteorological Station
Espolla	Espolla (SMC)
Roses	Roses (SMC)
Llivia	Puigcerdà (SMC) and ICGC Llívia (since June 2021)

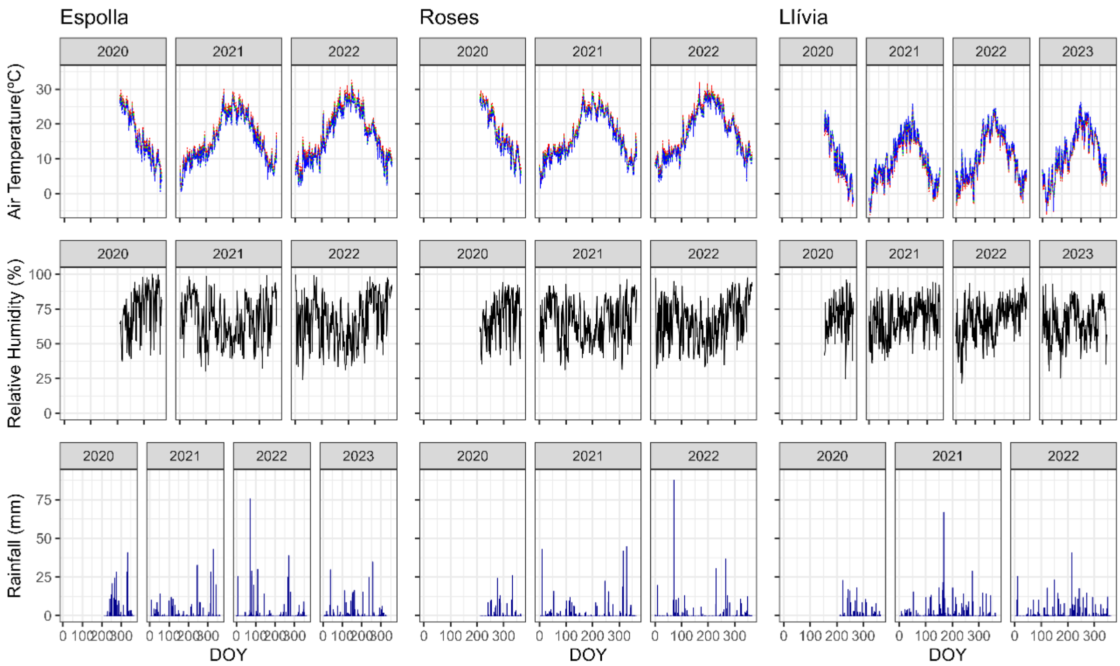


Figure 19. *Evolution of daily air temperature, air relative humidity and rainfall in Espolla, Roses and Llívia meteorological station from August 2020 to December 2023. The green line is air temperature, red line maximum temperature and blue line minimum temperature.*

The meteorological data is downloaded on a monthly basis at least. In Espolla station, there is no wind speed data, and for this reason we do not show wind speed data here. Regarding Puigcerdà station, we only have the precipitation data. We already have the other meteorological variables from SMC and from ICGC (Llívia station installed in June 2021). Figure 19 shows the evolution of air temperature, air relative humidity and rainfall in Espolla, Roses and Llívia sites. The rainfall for 2021 in Espolla, Roses and Llívia has been 339, 385 and 657 mm, respectively and for 2022, rainfall has been 417, 374 and 422 mm, respectively. The rainfall for the last month of 2023 remains to be updated (represented with an asterisk in Table 5). Nevertheless, in the first 11 months of 2023 it has only rained 288, 276 and 251 mm in Espolla, Roses and Llívia. In Espolla and Roses, the reduction in rainfall compared to the historical mean (1961-1990) is between -40 and -60%. In Llívia, rainfall for 2021 was +10% higher than the historical mean, but for 2022 the rainfall reduction was -30%. This 2023 is clearly dry, collecting a -60% less than the historical mean.

In Empordà pilots, Espolla is the site with more extreme temperatures, being 2023 clearly hotter (increasing Tmean in 1.6ºC in both sites) (Table 5). The Roses maritime influence allows to soften the maximum and minimum temperatures. Llívia mean temperature is around 6ºC bellow Empordà sites and 2023 has been also hotter (increasing Tmean in around 1.8ºC).

Table 5. *Summary of maximum (Tmax), minimum (Tmin), mean temperatures (Tmean), rainfall (PPT) and historical mean rainfall recorded in Espolla, Roses and Llívia for 2021, 2022 and 2023.*

	Espolla			**Roses**			**Llívia**		
	2021	**2022**	**2023**	**2021**	**2022**	**2023**	**2021**	**2022**	**2023**
Tmax (ºC)	39.5	40.2	42.4*	37.6	38.2	39.5*	34.6	34.1	35.4*
Tmin (ºC)	-5.6	-3.9	-4.1*	-3.8	-2.6	-2.6*	-12	-8.4	-10.4*
Tmean (ºC)	15.7	16.9	17.3*	16.5	17.7	18.1*	9.46	10.4	11.2*
PPT (mm)	339	417	288*	385	374	276*	657	422	251*
Historical mean PPT (mm) (1961-1990)	700.3			687.8			597.5		

**(the last month of 2023 remains to be uploaded).*

4. Preliminary results of the monitoring campaigns in vineyards in La Rioja

4.1. Monitoring results of the Soil

4.1.1. Soil characteristics

In La Rioja, samples have been obtained in mid-2021, together with microbial biodiversity analyses, following the same procedure as in Catalonia. Two locations were sampled (Clavijo and Tudelilla). This mid-2021 analysis was sent to Eurofins analysis laboratory, and we already have the results (Figure 20). For the long-established treatments in La Rioja, no resampling will be performed, and data will be compared between plots representing different conditions (Table 6).

***Table 6**. Soil samplings for soil physical and chemical properties and microbiologic diversity estimation along the project in the different sites in La Rioja. All samplings in the same year correspond to a single sampling period (about veraison): number indicates number of plots sampled.*

Site		2020	2021	2022	2023	Total
Tudelilla (LA RIOJA)		0	4	0	0	4
Clavijo (LA RIOJA)		0	4	0	0	4

Regarding soil characteristics in Clavijo, the control plots show higher organic matter (OM) and carbon (OC) than the cultivated plots; it is interesting to see, however, that they are higher in the terraced vineyards than in the slope vineyard. P are higher in the cultivated plots; K are slightly higher in the terraced plots and the terraced vineyard clearly shows the highest value of nitrates (Figure 20).
In Tudelilla, the oldest vineyard plot shows the highest P and K and, interestingly, also the highest values for OM and OC (higher than the control plot). The youngest vineyard also shows high values of OM and OC and the highest of N and other chemical elements (Cu, S, Ca…) (Figure 20).

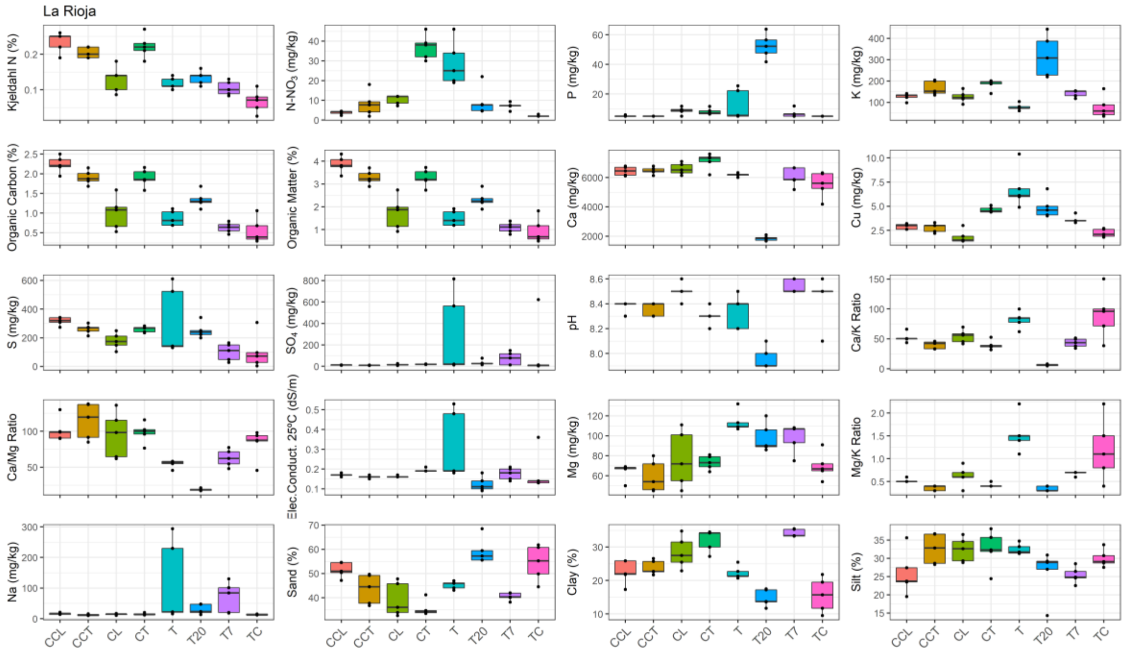


***Figure 20**. Box plot of soil characteristics variables in La Rioja plots. CCL represents Clavijo Control Slope, CCT Clavijo Control Terrace, CL Clavijo Slope, CT Clavijo Terrace, T Tudelilla, Young Vineyard, T20 Tudelilla Old Vineyard, T7 Tudelilla Medium Vineyard, and TC Tudelilla Control. The box portion of the box plot is defined by two lines at the 25th percentile and 75th percentile. The two whisker boundaries are the 5th and 95th percentile.*

Another analysed variable is soil texture, which is in general, sandy clay loam, clay loam and loam for Clavijo plots, and sandy loam, loam and clay loam for Tudelilla plots (Figure 21).

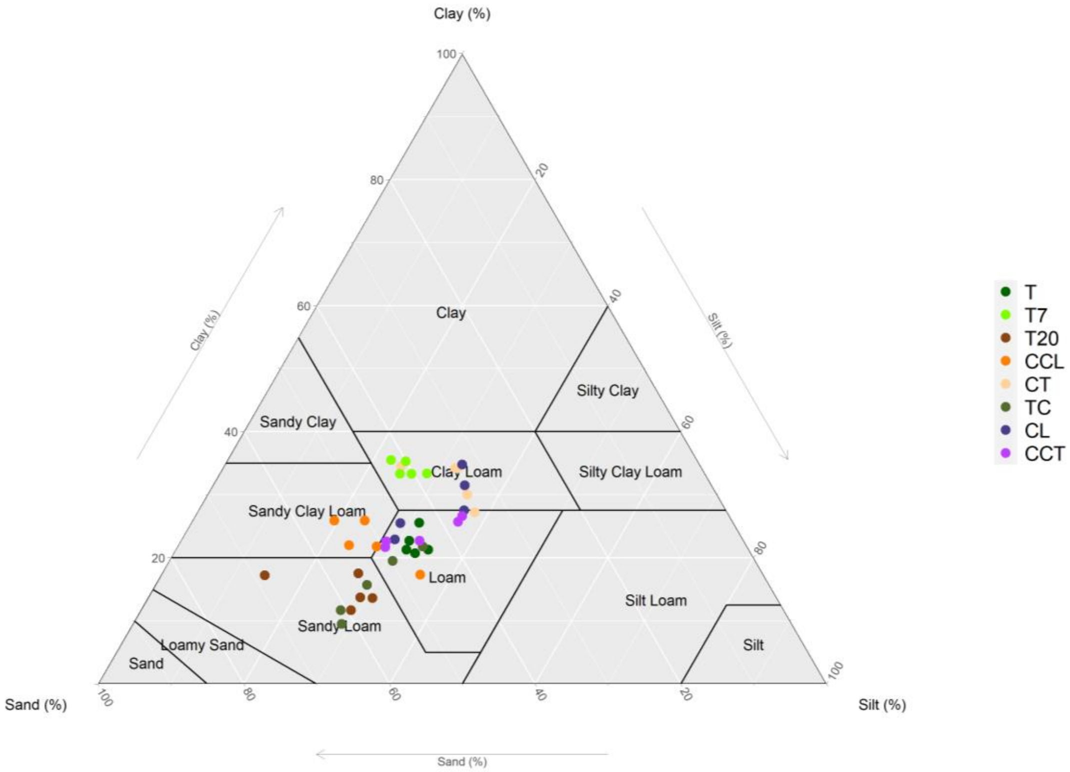


Figure 21. *Soil texture triangle with all the samples from Clavijo and Tudelilla plots. Different colours represent the different agricultural practices.*

4.1.2. Soil moisture

4.1.2.1. Tudelilla

In La Rioja, humidity probes were installed in two estates corresponding to two wineries, San Prudencio in Clavijo and Dinastia Vivanco in Tudelilla. In Clavijo, two vineyards are compared according to their position on slopes or terraces, while in Tudelilla, vineyards of different ages are compared. The original idea, in both cases, was that a difference is also made between vineyards with or without grass (Tillage), however, in Tudelilla this difference can no longer be made as there has been an error in the management of the land and the vegetation cover has disappeared in all the vineyards. Thus, in Tudelilla only the different ages can be compared.

The sensor network installed to monitor the evolution of the soil water content in the first 40 cm of the soil has had several problems mainly related on tasks in the field and on electronic problems.

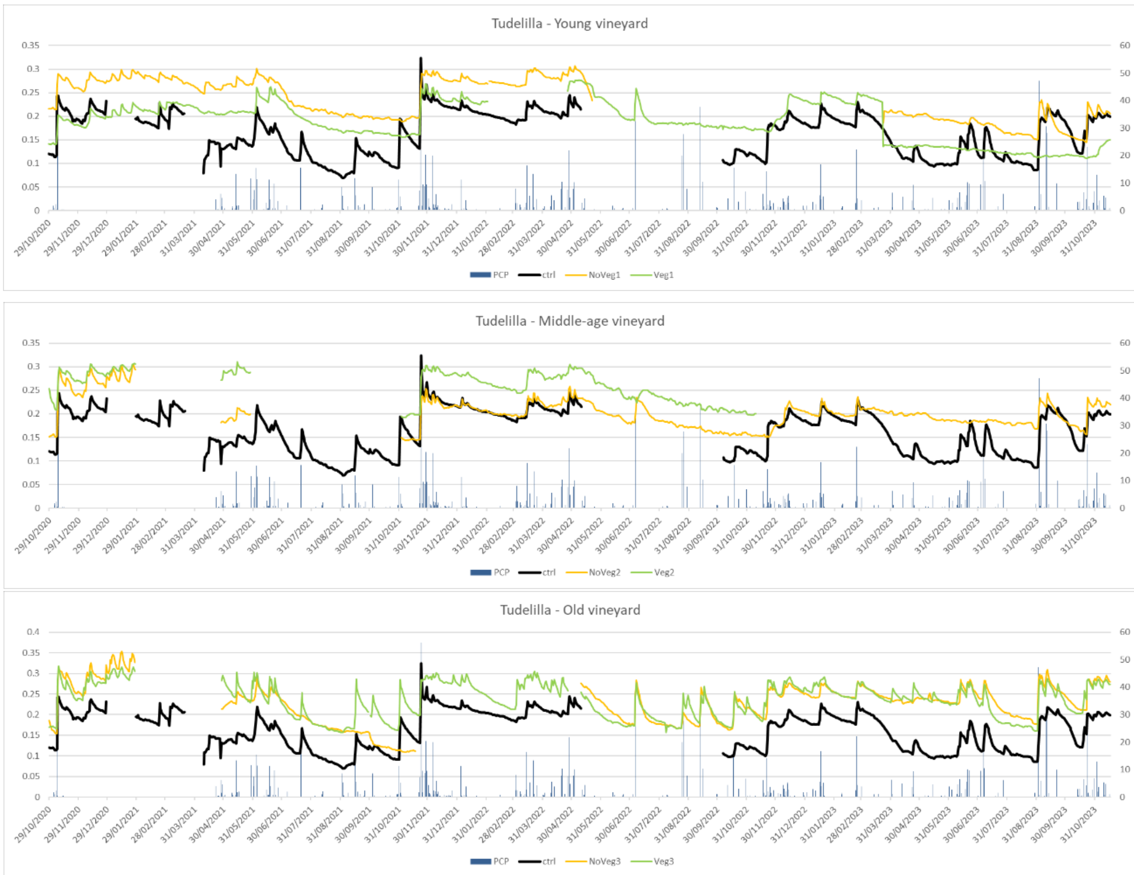


Figure 22*. Soil humidity and precipitation in vineyards plots (Tudelilla).*

Figure 22 shows the data collected by the sensor network in the three plots (young, middle-age and old vineyard) and in the control plot until mid-November 2023. Firstly, it can be seen how the data from the control plot, after several gaps, already mentioned in previous deliverables, stops in May for no apparent reason. It was started up in October 2022 and is not expected to cause any further problems. In the rest of the plots, the sensors are collecting data correctly, except in the case of the young vineyard, where several problems related to the work in the field (mainly pruning and ploughing) have been recorded. Rainfall has been updated with data from the winery owners' own station (CESENS). In general, there is a good relationship between rainfall and soil moisture.

Figure 23 tries to explain the seasonal distribution of soil moisture in the three study plots. This graph shows how the soil's capacity to retain moisture may be a function of age, as the oldest vineyard have the greatest capacity to hold water.

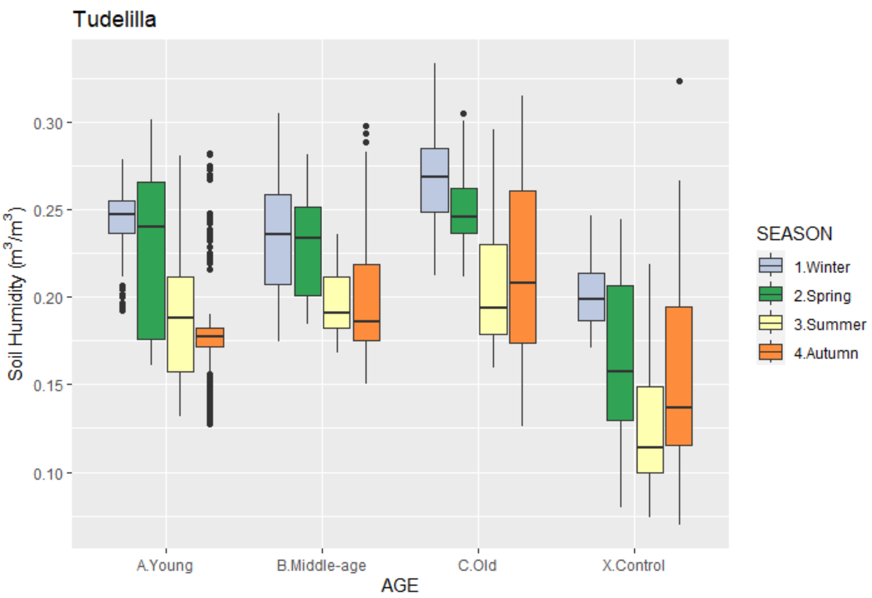


Figure 23. *Boxplot with seasonal soil humidity values in vineyard plots (Tudelilla)*

4.1.2.2. Clavijo

The sensor network installed to monitor the evolution of the water in the first 40 cm of the soil has been continuously recording since the installation, excepting some gaps explained below.

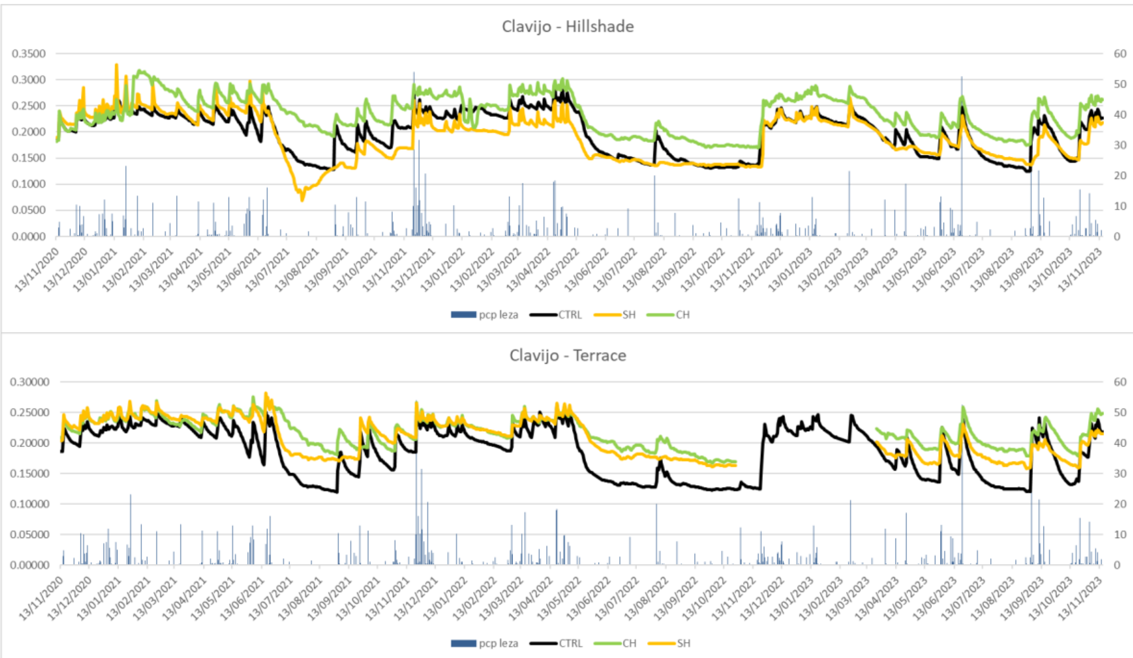


Figure 24. *Soil humidity and precipitation in vineyards plots (Claijo).*

Figure 24 shows complete series of soil moisture in the two types of plots in Clavijo, on hillslope and on terrace until mid-November 2023. In this case there have been no gaps, the humidity sensors have worked perfectly (excepting the one in Terrace, mentioned in previous deliverable). Although the graph with the difference between vegetation/non-vegetation treatment is maintained, this difference in treatment is only maintained on the terrace due to, as in Tudelilla, an error in the management of the vineyard, which the project team had nothing to do with. The precipitation data shown are from the SAIH A197 rain gauge at Leza and there is a good relationship between precipitation and soil moisture.

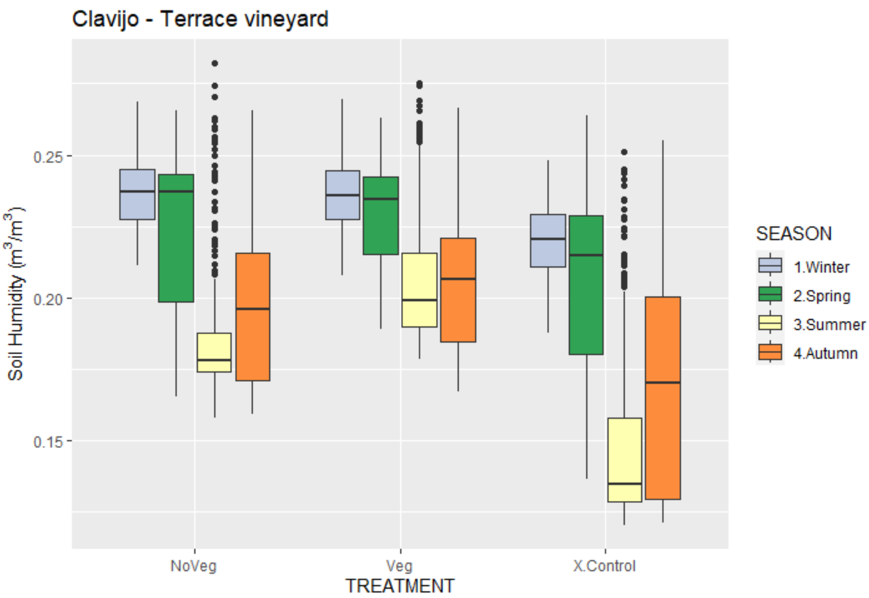


Figure 25. *Boxplot with seasonal soil humidity values in terraced plots (Clavijo)*

Figure 25 tries to explain the seasonal distribution of soil moisture in the terraced plots with the two treatments, with/without grass. In general terms it can be seen that there are few differences between the two treatments.

At the end of the project, when three full years of data are available, the data will be analysed, and conclusions will be drawn.

4.2. Monitoring results of Biodiversity

Sampling of soil for biodiversity was done in veraison 2021. Main results obtained revealed that:

a) Regional Geographic effect on microbial differentiation of diversity (beta diversity) was observed when Clavijo was compared with Tudelilla (F:8.72; $p<0.001$) (Figure 28).

b) The highest slope in the vineyards led to a decrease in final alpha and beta diversity in La Rioja. in: CL (H: 4.78) vs CT (H: 6.47) (F: 4.105 $p<0.001$). (Figure 27).

c) The slope (CL) at Clavijo was linked to a decrease in the abundance of Fungi and ammonium oxidizing bacteria and archaea (AOB and AOB), which was coincident with a loss of COT, CORG, clays and NTK (Figure 26).

d) Mantel Test revealed a correlation effect of: Slope (p=0.0001), clays (p=.056) and CaCO3 (p=0.0001), Cu (p=0.0033), Mg (p= 0.0112), pH (p=0.067) conditioning diversity changes in the soil.

e) Regarding microbiota composition, a lower prevalence of Verrucomicrobia and Acidobacteria was observed in La Rioja compared with Roses. The highest slope (CL) was coincident with a higher prevalence of Gemmatimonadetes (Figure 29).

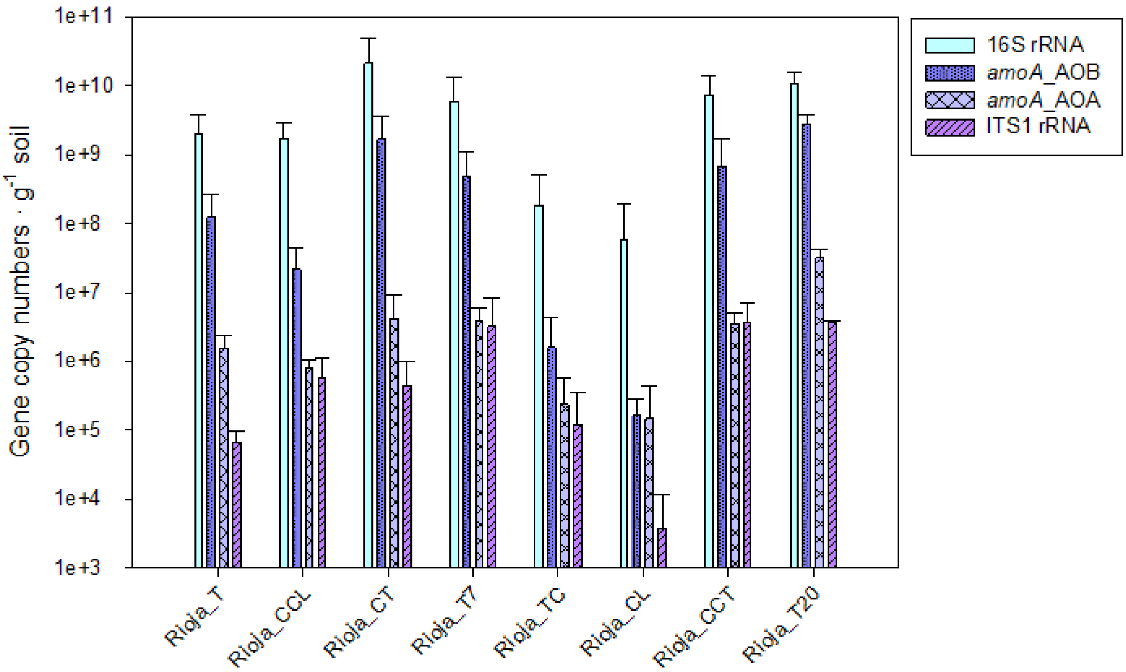
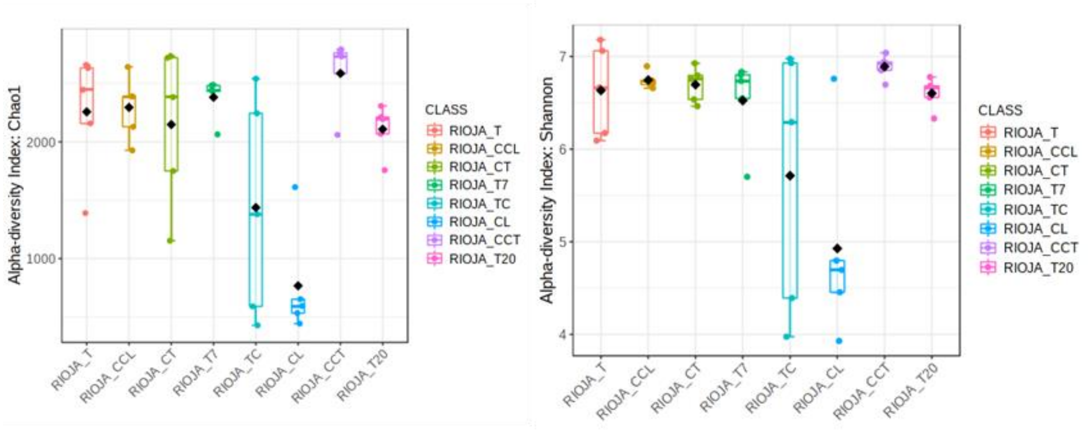


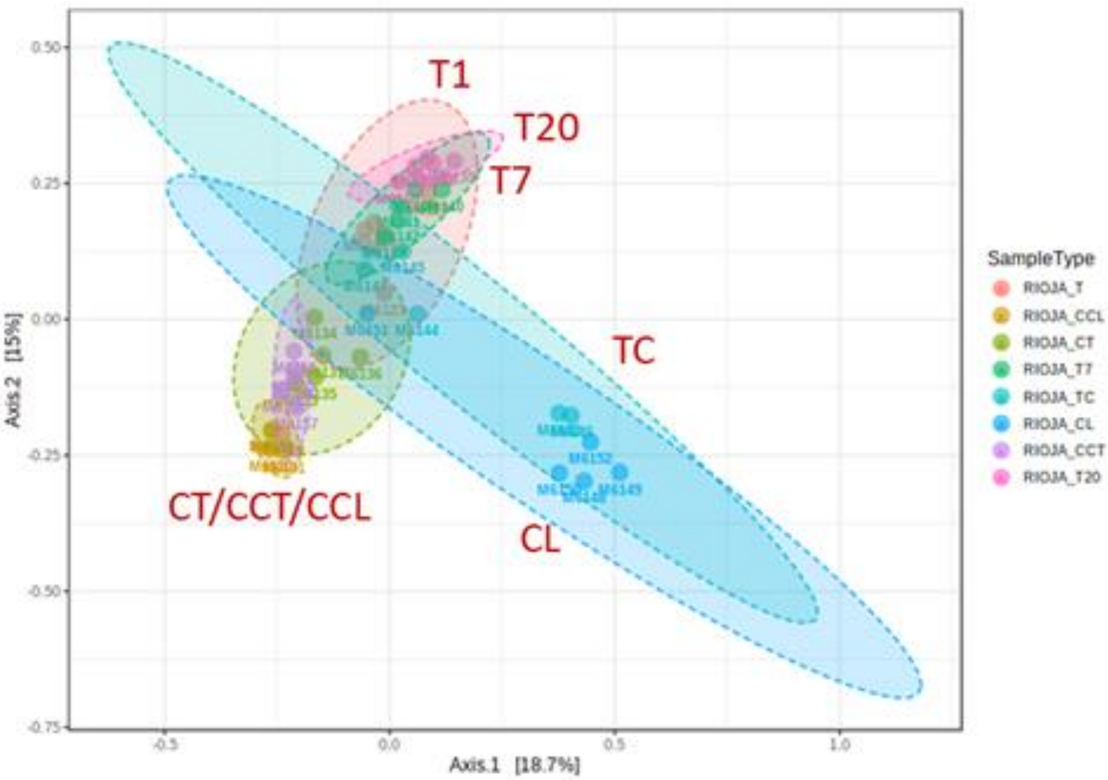
Figure 26. *Abundance of microbial biomass quantified by qPCR of gene populations in la Rioja soil (veraison sampling 2021). CCL represents Clavijo Control Slope, CCT Clavijo Control Terrace, CL Clavijo Slope, CT Clavijo Terrace, T Tudelilla, Young Vineyard, T20 Tudelilla Old Vineyard, T7 Tudelilla Medium Vineyard, and TC Tudelilla Control. Controls do not have vine plants. Total bacterial population (16S rRNA); AOB (ammonia oxidizing bacteria); AOA (ammonia oxidizing archaea); total fungal population (ITS1 rRNA). Presented values are the mean and SD of independent replicates (n=5).*



p-value: 0.012183; [Kruskal-Wallis] statistic: 17.953

p-value: 0.12107; [Kruskal-Wallis] statistic: 11.426

Figure 27. *Boxplots of Alpha diversity indexes (n=5/treatment), of richness (Chao1) and diversity (Sjannon) in Roses site (veraison 2021). CCL represents Clavijo Control Slope, CCT Clavijo Control Terrace, CL Clavijo Slope, CT Clavijo Terrace, T Tudelilla, Young Vineyard, T20 Tudelilla Old Vineyard, T7 Tudelilla Medium Vineyard, and TC Tudelilla Control. Controls do not have vine plants.*



[**PERMANOVA] F-value: 4.496; R-squared: 0.49584; p-value < 0.001**

Figure 28. *PCoA 2D ordination (Bray Curtis distance) revealing the effect of different crop management in the dissimilarity of microbial diversity structure in the vineyard soil at the Rioja site (-5 to -20cm). Permanova assessment revealed a significant geographic effect (Clavijo versus tudelilla), and slope vs Terrace at Clavijo on Bacterial diversity CCL represents Clavijo Control Slope, CCT Clavijo Control Terrace, CL Clavijo Slope, CT Clavijo Terrace, T Tudelilla, Young Vineyard, T20 Tudelilla Old Vineyard, T7 Tudelilla Medium Vineyard, and TC Tudelilla Control. Controls do not have vine plants.*

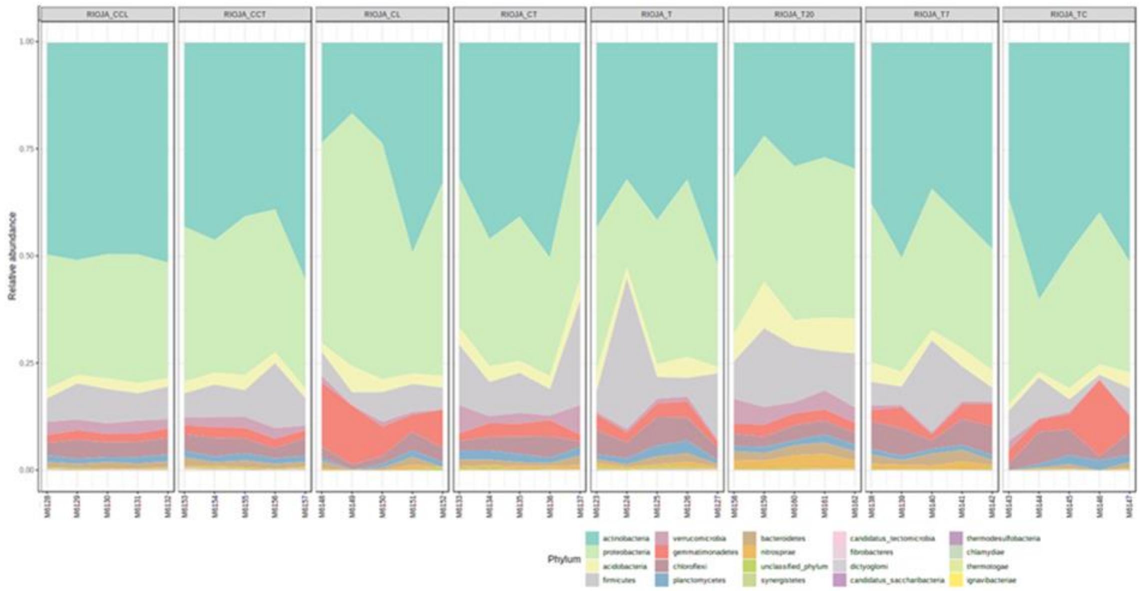


Figure 29. *Microbial community taxa distribution in vineyard soils in la Rioja (veraison 2021) (-5/-20cm) at Phylum level. Phyla >1% of relative abundance are reported. CCL represents Clavijo Control Slope, CCT Clavijo Control Terrace, CL Clavijo Slope, CT Clavijo Terrace, T Tudelilla, Young Vineyard, T20 Tudelilla Old Vineyard, T7 Tudelilla Medium Vineyard, and TC Tudelilla Control. Controls do not have vine plants.*

The most prevalent Fila in la Rioja soils were Actinobacteria and Proteobacteria (alpha), accounting for 75% of total relative abundance, followed by Firmicutes and Gemmatimonadetes. Interestingly, in la Rioja's vineyards, a lower prevalence of Verrucomicrobia and Acidobacteria was observed, compared to Roses site.

In La Rioja, the slope plots showed, in addition a lower alpha-diversity, a lower abundance of microbial population than on the terrace plots, confirming the potential effect of erosion and the loss of microbial populations in the soil. The results confirm the impact exerted by the edaphoclimatic context and the slope of the land on the microbial diversity of the vineyard soil. In Clavijo, a more negative impact of the slope on microbial diversity and abundance was observed, coinciding with a loss of clay, total organic carbon, and nitrogen, which could be associated with soil erosion processes.

4.3. Monitoring results of 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 regions.

For both sites (Clavijo and Tudelilla), we only have received grape production per hectare. In Clavijo, yield of hillslope is around 2000 kg ha^{-1} higher than terrace (Figure 30). There has been a reduction in the production during 2023.

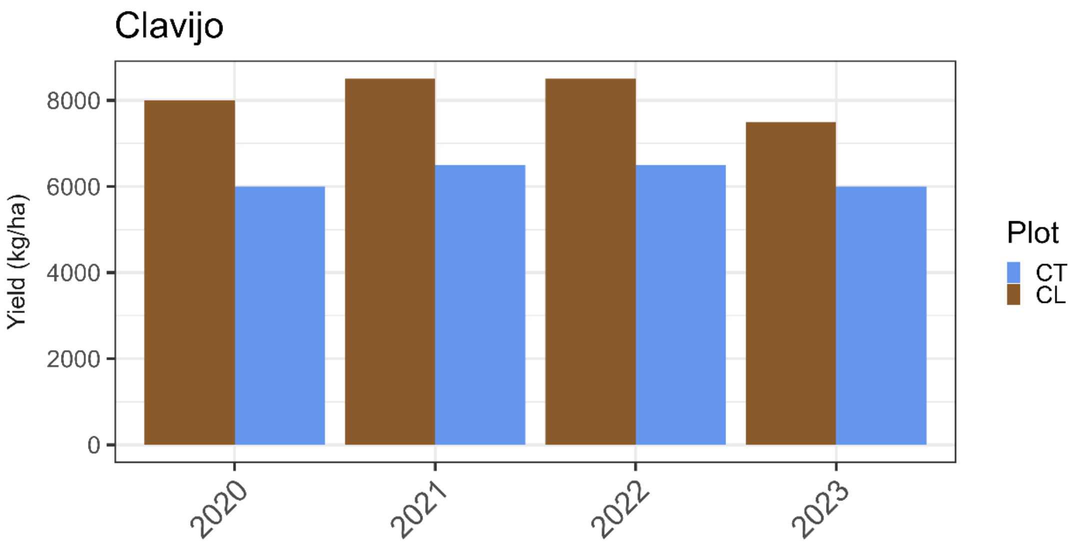


Figure 30. *Annual yield for Clavijo sites (CT: terrace and CL: hillslope)*

In Tudelilla, the yield is around 6000 kg ha^{-1} for all the monitoring years (2020, 2022 and 2023), except 2021 with 2000 kg ha^{-1}. The winemaker has only provided us 2022 data for each plot (T1, T7 and T20). The production in medium vineyard (T7) is higher (8000 kg ha^{-1}) than in young and old vineyard (around 6500-6000 kg ha^{-1}).

4.4. Monitoring results of Rainfall simulations

In La Rioja, the rainfall simulation experiments were carried out for humid and dry conditions in both Tudelilla (18 and 13 experiments, respectively) and Clavijo (14 and 12 experiments, respectively). In general, 3 replicates were performed per agricultural practice and site and condition. However, some results had to be removed because they were incorrect (e.g., RC>1 mm mm^{-1}). In some cases, more replicas were added to get more robust results.

In Clavijo under humid conditions (soil water content = 15%), only the vineyard in terrace produce runoff and sediment (2 experiments out of 4), however the response was very low (mean values of RC=0.09 mm mm^{-1}, SC=0.62 g L^{-1}, ER=1.73 g m^{-2}). The slope with shrubs presented the lower infiltration due to its dense vegetation cover. It is interesting to note that the vineyard in sloping field did not produce any runoff, even with very high rainfall intensities, due to its high infiltration capacity (Table 7; Figure 31).
Under dry conditions (soil water content = 9%), there was no hydro-sedimentological response in any of the studied plots. Again, the slope with shrubs presented the lower infiltration rates. With similar rainfall intensities, both vineyards (slope and terrace) and the shrubs in terrace show similar infiltration rates (Table 7).

Table 7. *Mean hydrogeological and sedimentological variables extracted from rainfall simulations in Clavijo under wet and dry conditions. RI: rainfall intensity (mm h^{-1}), IF: infiltration rate (mm h^{-1}), RC: Runoff coefficient (mm mm^{-1}), SC: Sediment concentration (g L^{-1}), ER: Erosion Rate (g m^{-2}).*

Site	Agricultural practice	RI (mm h^{-1})	IF (mm h^{-1})	RC (mm mm^{-1})	SC (g L^{-1})	ER (g m^{-2})
Clavijo Wet conditions	Hillslope control	28.8	14.3	0.00	0.00	0.00
	Terrace control	23.8	22.2	0.00	0.00	0.00
	Hillslope vineyard	58.2	39.1	0.00	0.00	0.00
	Terrace vineyard	39.6	21.2	0.09	0.62	1.73
Clavijo Dry conditions	Hillslope control	27.8	11.0	0.00	0.00	0.00
	Terrace control	36.5	30.4	0.00	0.00	0.00
	Hillslope vineyard	34.7	28.4	0.00	0.00	0.00
	Terrace vineyard	33.3	27.1	0.00	0.00	0.00

In Tudelilla under humid conditions (soil water content = 19%), it is interesting to observe that the hydrological response increases with the age of the vineyards, although the values remain low (mean values RC<0.10 mm mm^{-1}). Shrubs showed a similar response than the old vineyards, mainly due to the presence of patches of bare soil. In terms of sediment production, the medium and old vineyards produced slightly more sediment (although the values are also low, mean SC<0.5 g L^{-1} and ER<1.1 g m^{-2}) (Table 8).

Under dry conditions (soil water content = 6%), there was no hydro-sedimentological response in any of the studied plots, suggesting that all water was infiltrated or intercepted. Shrubs showed the lower value of infiltrated water, probably due interception by vegetation. The old vineyards showed the higher value of infiltrated water (Table 8).

Table 8. *Mean hydrogeological and sedimentological variables extracted from rainfall simulations in Tudelilla under wet and dry conditions. RI: rainfall intensity (mm h^{-1}), IF: infiltration rate (mm h^{-1}), RC: Runoff coefficient (mm mm^{-1}), SC: Sediment concentration (g L^{-1}) and ER: Erosion Rate (g m^{-2}).*

Site	Agricultural practice	RI (mm h^{-1})	IF (mm h^{-1})	RC (mm mm^{-1})	SC (g L^{-1})	ER (g/m^2)
Tudelilla Wet conditions	Scubland (control)	20.3	12.6	0.07	0.25	0.17
	Young	30.7	16.9	0.00	0.00	0.00
	Mid-term	34.3	21.4	0.3	0.44	1.08
	Old	40.5	12.7	0.06	0.24	0.46
Tudelilla Dry conditions	Scubland (control)	23.0	16.6	0.00	0.00	0.00
	Young	25.4	18.0	0.00	0.00	0.00
	Mid-term	21.4	20.7	0.00	0.00	0.00
	Old	34.1	31.4	0.00	0.00	0.00

4.5. Monitoring of site meteorological conditions

The registration of the meteorological conditions is key to understand the evolution of previous variables along the project duration. With this objective, we have installed air temperature sensors and relative humidity sensors to record in continuum these meteorological variables.

4.5.1. Tudelilla

Meteorological conditions are being recorded continuously since 29-10-2020. Three Temperature/Relative Humidity sensors were installed, one in each vineyard. In this case, it has not been necessary to install a rain gauge because we have the data recorded by an own station of the winery (CESENS).

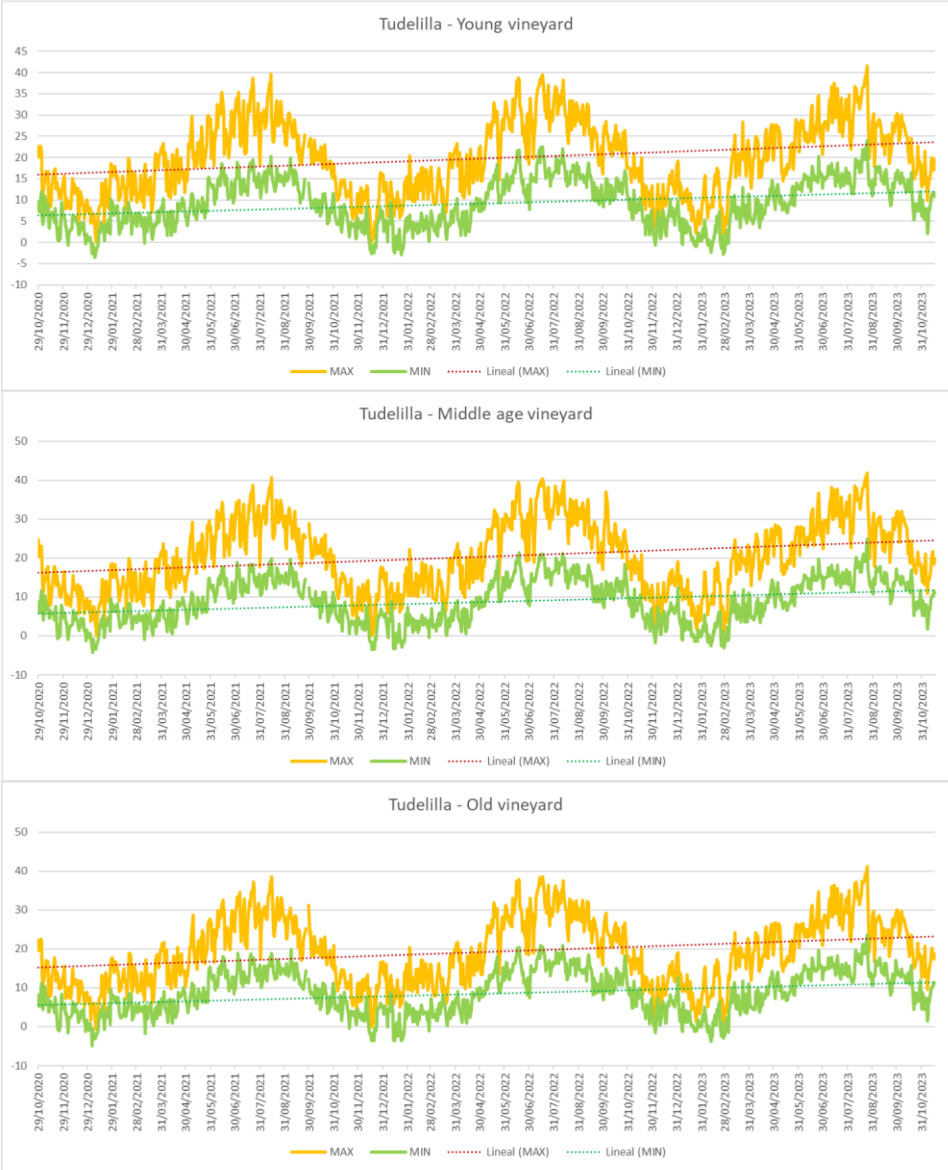


Figure 31. *daily maximum and minimum temperature data from the three thermometers installed in Tudelilla.*

Figure 31 shows the daily maximum and minimum temperature data from the three thermometers installed. Table 9 summarises the data obtained, where we can see how the maximum temperatures are very similar between the three vineyards given their proximity, although it could be considered that the middle-aged vineyard has the most extreme conditions, with a greater range of temperatures between -4.3ºC and 41.9ºC although the minimum temperature was recorded in the old vineyard with -4.9ºC in January 2021.

Table 9. *Summary of maximum and minimum temperatures recorded in Tudelilla.*

	Young		Middle-age		Old	
	Tmax	Tmin	Tmax	Tmin	Tmax	Tmin
Max	41.7	24.1	41.9	24.9	41.1	23.5
Min	0.2	-3.6	0	-4.3	-0.6	-4.9
Mean	19.8	9.1	20.4	8.7	19.2	8.5

4.5.2. Clavijo

Meteorological conditions are being recorded continuously since 10-11-2020. Three Temperature/Relative Humidity sensors were installed, one in each vineyard (hillslope, terrace and an additional one under a tree on the hillslope which disappeared on 6 October 2022). In this case, it has not been necessary to install a rain gauge because we have the data recorded by the A197 “Leza” del SAIH (Servicio Automático de Información Hidrologíca del Ebro).

Figure 36 shows the daily data of the maximum and minimum temperatures of the three thermometers installed. Table 10 summarises the data obtained, where we can see how the maximum temperatures are very similar between the 2 vineyards given their proximity, although it could be considered that the hillslope has the most extreme conditions, with a greater range of temperatures between -4.7ºC and 41.3ºC.

Table 10*. Summary of maximum and minimum temperatures recorded in Clavijo.*

	Terrace		Hillslope	
	Tmax	Tmin	Tmax	Tmin
Max	39.3	23.8	41.3	23.9
Min	-1.2	-4.5	-1.8	-4.7
Mean	18.4	8.4	17.9	8.3

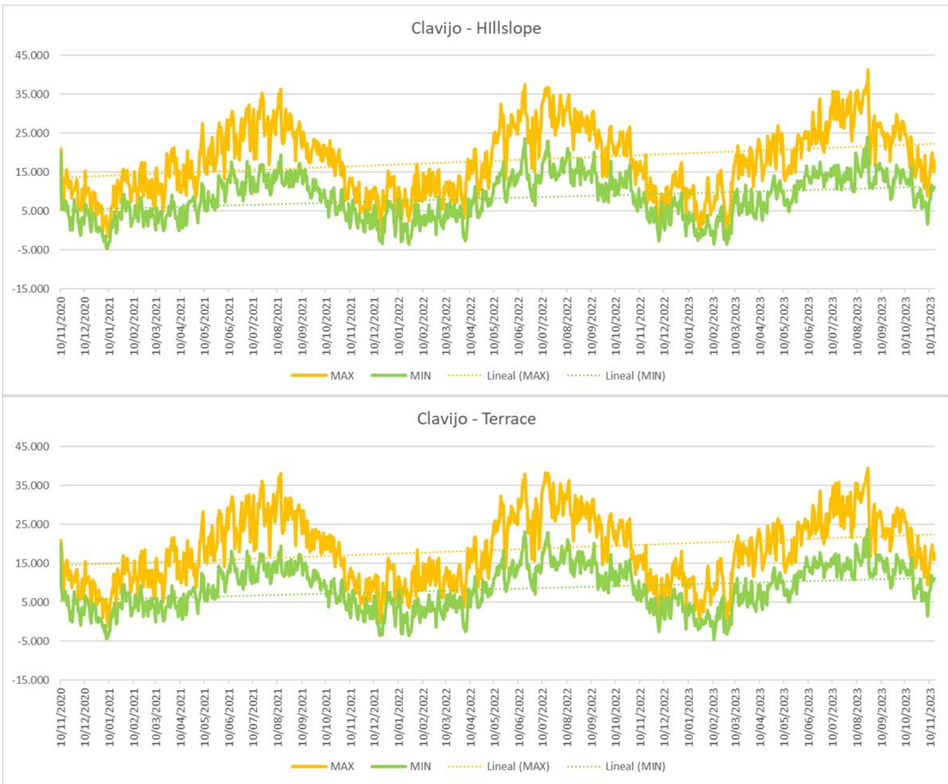


Figure 32. *Maximum and minimum temperatures in experimental plots (Clavijo)*

5. Conclusions

The main objective of this deliverable is to present the three **first years monitored results of the implementation C3 action**.

The preliminary results of physical and chemical soil properties and soil moisture, soil microbial biodiversity; rainfall simulations and meteorological conditions, are shown to analyse the effects of adaptive vineyard establishment and agronomic practices. However, to draw strong conclusions, data should be thoroughly analysed considering all the variabilities that exists within the plots including the different viticultural managements applied in each plot and treatment.

Finally, it should be highlighted that all the monitoring tasks planned during these three years have already been developed and data has been analysed or, in some cases, is being analysed by the different project partners. The monitoring of the pilot experiences has been made from summer of 2020, and they are scheduled until December 2023. Except for soil biodiversity, with a schedule to analyse the data during the last 2 years of the project. In addition, crop productivity is the only variable not yet achieved completely, as it depends on vineyard owners. Consequently, **all the activities** (except crop productivity in La Rioja) **and the periodicity defined in the LIFE MIDMACC proposal have been successfully completed**.

	Vineyards in Catalonia	
Soil	**Soil characteristics**	In **Espolla**, NC plot shows higher values of macronutrients (N-P-K), organic carbon and organic matter than WE and CM plot. NC soils have high values of macronutrients and organic matter. Therefore, the soil impact in terms of nutrients availability of a new spontaneous cover crop is lower than a soil conventional management, which presents lower values of these nutrients. In the case of a spontaneous cover crop allowed for several years (WE), the behaviour is quite similar than CM. In **Llívia**, VNV has higher macronutrients than NV and similar organic carbon and organic matter. Pastureland shows higher values of organic carbon, organic matter, and N. In **Roses**, there are no differences between the adaptation measures in terms of macronutrients, except for GT for Kjeldahl Nitrogen, with lower values than the rest of the plots. Organic carbon and organic matter are also lower in GT than the other plots. For scrubland, considered as the control, the values of Cu and SO_4 are lower, and for the contrary, the values of Na are higher than the rest of the plots. In TS the values of Mg, SO_4 and Na are lower. Soil texture is in general sandy loam and sandy clay loam for all **Espolla** plots, sandy clay loam for **Llívia** vineyards, except in pasture which is clay loam, and Sandy Loam for **Roses** plots.
	Soil moisture and temperature	For 2021, in **Espolla** pilot NC presents an intermediate state between WE and CM, responding similarly to CM in autumn but quickly reaching similar SWC to WE, then following the same evolution till next spring, with CM presenting lower values along autumn and winter. In late winter vegetation activation can be deduced from a decrease in SWC in all plots, although much slower in CM, which may be attributed to the absence of green cover. Sensibility to spring rains is again intermediate for NC, which joins SWC evolution of CM by the end of spring till next autumn, when the cycle repeats, which might be related to a still poorly developed green cover. In contrast, for 2022 WE and NC present throughout the whole year higher SWC values than CM, even in late winter. In the **Roses** pilot, no clear pattern could be related with these conditions. The two plots presenting the lowest values shared no common factor: trellis + slope (TS) and Gobelet + terrace (GT). Both terraces seem to be more sensitive to spring rains as slope vineyards only respond near the soil surface. Gobelet + Slope (GS) is the least sensitive to spring and summer rains and keeps higher SWC most of these seasons. In the **Llívia** pilot, deepest sensor is still sensitive to rain events all over the year and SWC is always highest at this depth, revealing small water capture by vines. In the new vineyard, higher canopy development, the presence of a spontaneous green cover and a straw mulching in the vine row result in a slower SWC dynamic, not so sensitive to rains but conserving more soil water in spring and most of summer, even with presumably a higher water extraction by vines.
	Soil Microbial diversity and abundance	**In Espolla**, WE has a significant lower Richness (Chao1 3000) and diversity (H: 6,65) than soil with young cover (NC) (Chao; 3700, and H: 7,15). WE has a lower relative predominance of Actinobacteria (25% vs 30% NC) and higher predominance of Acidobacteria (28%WE vs 20-25%NC), and Firmicutes (6-8% WE vs 2-4% in NC), and a similar prevalence of Proteobacteria (20-25% in WS and NC) and Verrucomicrobia (3-6%) The

	Vineyards in Catalonia	
		age of cover crops impacted in microbial diversity (Bray Curtis dissimilarity, Permanova F: 6,205 P<0.01). **In Roses** the presence of vine was linked to a higher richness and diversity (Chao1 and H index of 4700-5500 and 7,6-7,7 in vineyards vs Chao of 4100 and H 7,2 in Scrubland). The results revealed the positive effect of vine plant to boost microbial diversity and richness in soil in Roses site. Main predominant Phyla in Roses were Actinobacteria (37,4±2,5%, higher than in Espolla (25-30%), Proteobacteria (25,5±2,0%) (class α), Acidobacteria (9,5±1,3%, clearly lower than in Espolla (25-30%), Firmicutes (6,9±1,7%), Verrucomicrobia (8,7±2.1% higher than in Espolla). Significant difference in diversity (ASVs level) due to vine plant presence, and also in slope plots compared with terraced plots (Permanova F: 5,318 p<0,001). **In Llivia** diversity results will be presented at the beginning of 2024 combining processed data from sampling campaigns performed in 2020 and 2023. **Regarding prokaryote and fungal abundance**: In Espolla and Llivia both vineyards have similar abundance of total bacterial populations (10^8-10^9 16S rRNA gene copy numbers/g sample), but in general Espolla shows less total abundance. Espolla presents a highest ratio of fungal/bacteria population in all samples. Ammonia oxidizing prokaryotes are present in all samples of both sites. In Roses a higher bacterial abundance is revealed (10^9-10^{10} 16S rRNA gene copy numbers/g sample), high renge of AOB (10^8 amoA copies/g), but lower fungal abundance (10^5-10^6 ITS copes/g vs 10^7-10^8 ITS copies/g) than observed in Espolla and Llivia. The three sites encompass a similar AOA abundance (10^6 copies *amoA*/g).
Vineyard production	**Total grape production, grape, and wine quality**	In **Espolla**, yield of CM is higher than NC and WE. The lower values of alcoholic strength are showed by CM and the highest by WE, with NC presenting intermediate values. In **Roses**, there are no significant differences in production between the different practices. Although, the production in trellis plantation is slightly higher than in gobelet, as expected. Alcoholic (ABV) strength present the reverse pattern, with higher values in gobelet pilots than terrace pilots. In **Llívia**, yield is improving with time as expected on a young vineyard.
Rainfall simulation	**Hydrological response and soil erosion**	In **Espolla** CM plot, the runoff coefficient is higher than the NC and WE plots. In parallel, the infiltration coefficient in the 2021 dry campaign shows higher values for CM and lower values for WE. This pattern is reversed in 2020 and 2022 wet campaigns. If the soil is wet, there are no significative differences in the IC among management practices. If the soil is dry (as happened in 2021), IC is higher in CM than WE, NC, and F. Another dry campaign would be needed to confirm this pattern. The sediment concentration in the runoff water does not vary too much between plots, although the variability is greater in the WE and the F, and this is reflected in the erosion rate. We find that, in correspondence with what happens with runoff, the erosion rate is higher in the CM plot, in the 2022 campaign above all. Also, the NC has higher erosion values than the WE, which has similar values than F, but without statistical differences. In **Roses**, in general there are no statistical differences in the hydrogeomorphological responses.

	Vineyards in Catalonia	
		In **Llívia**, the runoff was between 12 and 27 mm in the P plot and, on the other hand, the VNV, with a bare soil, the rates are up to 4 mm. In the VNV, there was a straw cover, manually added, and almost no vegetation. In the NV, the runoff was very low or practically non-existent. There is a high range in the erosion rate in the VNV, while the erosion rates in the NV are very low. In the case of P, the erosion rate is very stable between measurements. If we look at the data from the point of view of sediment concentration, the P cover seems to protect very well from erosion, and remarkably high runoff resulted in minimal erosion that can exceed the NV one, which it has a lower erosion rate. But, due to the much lower runoff, and not to the concentration of sediment, which is higher than in the P and similar with the VNV.
Site meteorological conditions	**Meteorological variables**	Meteorological variables are continuously recorded since June 2020

	Vineyards in La Rioja	
Soil	**Soil characteristics**	In **Clavijo**, the control plots show higher organic matter (OM) and carbon (OC) than the cultivated plots; they are higher in the terraced vineyards than in the slope vineyard. P are higher in the cultivated plots; K are slightly higher in the terraced plots and the terraced vineyard clearly shows the highest value of nitrates. In **Tudelilla**, the oldest vineyard plot shows the highest P and K and the highest values for OM and OC. The youngest vineyard also shows high values of OM and OC and the highest of N and other chemical elements.
	Soil moisture and temperature	In **Tudelilla**, the seasonal distribution of soil moisture in the three study plots with the two treatments, grass/no gras, present no clear pattern. At the end of the project, when three years have been completed, a comprehensive review of the results will be carried out. In **Clavijo**, the seasonal distribution of soil moisture in the two study plots with the two treatments, with/without grass show fewer differences between the two treatments, while on the slope the vegetation treatment is able to retain more soil moisture, perhaps due to the slope of the plot. At the end of the project, when three full years of data are available, the data will be analysed, and conclusions will be drawn
	Soil Microbial Biodiversity	**Clavijo and Tudelilla**: A regional geographic effect on microbial differentiation of diversity (beta diversity) was observed when Clavijo was compared with Tudelilla (F:8.72; p<0.001). The most prevalent Fila were Actinobacteria (35-50%, higher than in Catalonia's sites) and Proteobacteria (alpha), accounting for 75% of total relative abundance, followed by Firmicutes and Gemmatimonadetes. Interestingly, in la Rioja's vineyards, a lower prevalence of Verrucomicrobia and Acidobacteria was observed compared with Roses site. Mantel Test revealed a correlation effect of: Slope (p=0.0001), clays (p=.056) and CaCO3 (p=0.0001), Cu (p=0.0033), Mg (p= 0.0112), pH (p=0.067) conditioning diversity changes in the soil. Lower alpha diversity and richness when compared with Roses site, and similar with Espolla. **In Clavijo**, the increase in slope in the vineyards led to a decrease in final alpha diversity CL (H: 4.78) vs CT (H: 6.47), also generating beta diversity dissimilarity (F: 4.105 p<0.001). The alpha diversity ranged H 6,7-6,9 except

	Vineyards in La Rioja	
		for slope parcels (CL) with H: 4,9. The slope was also linked to a decrease in the total abundance of Fungi and ammonium oxidizing bacteria and archaea (AOB and AOB), which was coincident with a loss of COT, CORG, clays and NTK, and an increment in the relative abundance of Gemmatimonadetes (5-10% vs 2-3% in the other plots). **In Tudelilla**, a homogeneous alpha diversity (Chao1 2100-2500 and H 6,6-6,7) was observed when vine plant was present. Without vine plants the microbial diversity was lower (Chao1 1500, and H: 5,8)
Vineyard production	**Total grape production, grape, and wine quality**	At this moment, we have requested this information from the vineyard owners, but they have not yet provided it to us. We expect to receive this data throughout 2023.
Rainfall simulation	**Hydrological response and soil erosion**	In La Rioja, the rainfall simulation experiments were carried out for humid conditions (2020) for both Clavijo and Tudelilla, and for dry conditions (2022) in Tudelilla. Although 3 experiments were performed per agricultural practice type, some results had to be removed because they seemed incorrect. This can be due to problems in either the rainfall simulation experiment (e.g., the circular ring is not correctly fixed in the ground) or the post processing of the water samples. In **Clavijo** under humid conditions, only the vineyard in terrace produce runoff and soil erosion. These data are not sufficient to withdraw consistent conclusion so an additional campaign under humid conditions will be performed. In **Tudelilla** under humid conditions, the hydrogeomorphological response was low, except for the mid-term vineyard that showed higher erosion, probably due to the steeper hillslope gradient. The young vineyard did not produce runoff nor sediment. Under dry conditions, only the old vineyard produce runoff, with a low RC of 0.04, and sediment, with higher values than under humid conditions.
Site meteorological conditions	**Meteorological variables**	Meteorological conditions are being recorded continuously since November 2020

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