Impact of long term agroecological and conventional practices on subsurface soil microbiota in Macabeu and Xarel·lo vineyards

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Abstract

The impact of organic versus conventional practices on the soil microbial diversity, and its relationship with soil physicochemical properties in the subsurface depth is not well understood. Soil bacterial diversity is an important contributor towards plant health, productivity, and stress response. A field experiment was conducted in commercial vineyards with long-term implementation of agroecological (ECO) and conventional (CONV) management (>10 years) to determine the effect of management on the soil bacterial community, at Penedès (Catalonia). ECO practices did not affect bacterial and fungal abundance but increased the ammonium oxidizing bacteria and alpha-diversity (Simpson index) changing taxa composition. There was a combined effect of the management and plot on beta diversity and on the soil microbial community structure (ASV distribution and abundance). Main phyla were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Gemmatimonadetes* whose relative abundances were affected significantly by the management (excepts *Acidobacteria*). Differential abundance analysis in ECO revealed an increase of *Proteobacteria*, and decrease of *Actinobacteria*, *Gemmatimonadetes* and *Firmicutes* phyla. The variety did not affect the soil microbial community structure overall, but management affected the diversity in each variety. Mantel-test revealed significant effect of physicochemical parameters (texture, EC, OOM, $SOC_{topsoil}$/$SOC_{subsurface}$, fungal abundance, and AOA abundance) on the soil microbiota structure.

Introduction

The effect of soil and crop quality in the Terroir concept has been well documented over the last 30 years, especially with respect to grapes variety used in wine production (Vaudour, 2002; Vaudour et al. 2015). Other complementary factors have been well documented i) the vine rootstock, ii) water availability, iii) climate, iv) soil properties, and viticultural-oenological practices (Van Leeuwen, Roby, & de Rességuier, 2018; Van Leeuwen & Seguin, 2006). However, the potential role of soil microbiota in vineyards to the Terroir, and its linkage on the soil properties in the vineyard (i.e soil organic carbon, soil carbon storage and sulphur cycle) has only been reported until recently, mainly at top soil zone (Bokulich et al. 2014; Gilbert et al., 2014; Knight et al., 2015; Miura et al., 2017; Mocali et al., 2020; Zarraonaindia et al., 2015). Few studies have been reported focusing on the effects of the comparing different crop management and fertilization strategies, including soil characteristics from the vineyards in the assessment (Burns et al., 2016; Koeberl et al., 2020; Longa et al., 2017; Unc et al., 2021). Recently, a global microbiome assessment has revealed the high diversity and the similarity of the distribution of the main microbial taxa in the topsoil (first 10-20 cm of soil) in different winegrowing regions (Gobbi et al., 2022). In the topsoil, the higher organic matter content has an important role driving the final diversity of the microbial populations in top soil zone, but the proximity of bulk soil to the rhizosphere depth (-20 to -40 cm) is omitted, where the effects of crop management and organic matter content are not well understood, but the microbiota diversity could be highly relevant for the plant.

The aim of this study is to assess the effect of different long-term managements (conventional vs agroecological) on the subsoil bacterial diversity, and on the size of the bacterial and ammonium-oxidizing populations (bacteria and archaea) in commercial vineyards and its relationship with key subsoil (-20/-40 cm) physicochemical parameters as main drivers of the subsoil microbiota diversity.

Materials and methods

Location of the case study, vineyard plots and crop management strategies

As a case study to compare the effects of agroecological sustainable vineyard practices on the soil quality and microbiological diversity, 6 vineyards were selected in 3.5 km radius from Sant Sadurní d'Anoia (Alt Penedès Catalunya): 3 organic (ECO3, ECO8 and ECO12) and 3 conventional (CONV1, CONV2, CONV3) vineyards were sampled in April 2021. All vineyards were small plots (<2.5 ha), older and with continued type of management (ECO/CONV) for more than 10 years. Regarding to management practices, only CONV3 applies herbicide, whereas the other 2 conventional growers use only mechanical weed control. The ECO vineyards use a combination of cover crops, mowing and summer tillage, with occasional grazing or horse-powered tillage. Fertilizer is organo-mineral (4-6-10 30% OM) for the CONV plots, whereas in ECO was a composted cow manure and biodynamic amendments (last input was 4 months before the sampling). The pruning wood is usually left in all plots (ECO and CONV) on the ground and incorporated to the soil with the first tillage of the year. The mean yield during the last 3 years is 6,548-8,697 kg/ha in CONV and 8,079-9,194 kg/ha in ECO.

Soil collection and physicochemical characterization of soil:

For every selected point (5/vineyard), 4 soil drills were taken around a grapevine (40-80 cm from the trunk and at S, N, W, E directions. All the 4 cores for each depth (-5/-20cm and -20/-40 cm) around a vine were homogenized to form composited sample, chilled to 4/8 °C during the sampling time, kept at −80°C in the lab for further microbial analyses and air dried for further physicochemical analysis. Soil carbon content (SOC) and oxidizable organic matter (OOM) were analysed in -5 to -20 cm, whereas a complete physicochemical assessment was performed for samples collected from -20 cm to -40 cm, which were sent to analyse to a reference laboratory: soil texture, Soil Organic Content (SOC) (% of dry matter), Oxidizable organic matter (% of dry matter), Nitrogen Kjeldahl (% of dry matter), N-NO_3^-, N-NH_4^+, P (Olsen), Ca^{2+}, Mg^{2+}, K^+, Na^+, pH, Electric Conductivity (EC, dS/m), S, SO_4^{2-}, Fe, Cu, Mn, Zn and Mo. All statistical analyses were carried out with R Statistical Software version 4.1.1. We used mixed-effects models (package nlme, R Core Team, 2021), specifying reply as a random factor, to analyse crop management differences on soil physicochemical parameters.

Microbial community assessment of soil samples

DNA extraction of soil material from each composite sample (n=30), was performed by using PowerSoil™ DNeasy Isolation 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). To assess the bacterial diversity of soil samples, 16S rRNA amplicon (V3-V4 region) gene libraries were sequenced in a MiSeq equipment. Primers were removed from the demultiplexed fastq files by using Cutadapt software, and the paired reads were filtered and trimmed, denoised and merged using the R package DADA2. The taxonomic affiliations of the ASVs were assigned by using the naïve Bayesian classifier method, using the RDP and Greengenes database, and compiled into each taxonomic level.

To assess alpha diversity, Shannon (H), Inverted Simpson (I/D), Richness (Sobs) and Chao 1 indexes, were calculated in rarefied samples by using the Mothur software (version 1.46.1). The dissimilarity in overall community composition among samples (beta diversity) were calculated with Bray–Curtis distance and ordinated in PCoA. The contribution of each management crop practice to change the microbial community structure were assessed by means of non-parametric PERMANOVA and by analysis of similarity ANOSIM, of total ASVs rarefied distributions, in Vegan R package. Differential abundance testing of representative ASVs and taxa was performed by conducting basic univariate tests with two-group comparison, Wilcoxon tests, and adjusted p-values by standard False Discovery Rate (FDR). The contribution of environmental data to microbial community structure (ASV level) were tested by means of Mantel tests (Vegan R package).

Results and discussion

Physicochemical characteristics of soil and their influence on the microbial diversity

Even though there is an internal variability among vineyards, the differences between managements have been revealed (Table 1), in terms of accumulation of organic matter (OOM) and soil organic carbon (SOC) in the topsoil (-5/-20 cm) and in the subsurface (-20/-40cm). Vineyard soils were alkaline (pH 8.4), with a medium conductivity (0.15-0.16 dS/m), low content in OOM (1.04-1.64%) and SOC (0.42-0.61%). The soil texture of the plots ranged from silty clay loam to sandy loam, being this heterogeneity quite normal in agricultural soils. In general, ECO vineyards showed a higher content (p=0.001) in clay (24%) than CONV (17%), whereas CONV has a higher content (p=0.03) in sand (46% CONV and 34% ECO). It is noteworthy that the effect of soil texture contributing changes in the microbial community (ASVs distribution) was significant (ANOSIM, R: 0.3419, P: 0.0022; Mantel Test clay/silt/sand R: 0.378, P: 0.0001). The dispersion of soil texture in the vineyards could highly conditionate the interpretation of managements effects on the diversity of microbial communities.

Table 1. Significant physicochemical parameters of soil (-20/-40cm) comparing ECO and CONV management strategies. Statistical tests for the significancy of each parameter, and Mantel Test to determine its correlation with changes in the microbial diversity (ASVs distribution) are enclosed.

	CONV mean ± SE	**ECO mean ± SE**	**ANOVA p-value**	**Mantel Test Statistic**	**Mantel Test (p-values)**
SOC 20-40 cm (%)	0.42 ± 0.06 a	0.61 ± 0.09 b	**0.042**	0.118	0.099
Diff SOC (%) (top-sub)	-0.04 ± 0.04 a	0.24 ± 0.05 b	**0.000**	0.141	0.051
Ratio SOC (5-20)/(20-40)	0.99 ± 0.09 a	1.61 ± 0.13 b	**0.000**	0.216	**0.0007**
OOM 20-40 cm (%)	1.04 ± 0.11 a	0.73 ± 0.11 b	**0.043**	0.149	**0.045**
Diff OOM (%) (top-sub)	-0.02 ± 0.06 a	0.44 ± 0.07 b	**0.000**	0.141	**0.039**
Ratio OOM	1.07 ± 0.08 a	1.64 ± 0.12 b	**0.000**	0.201	**0.007**
pH	8.43 ± 0.02 a	8.43 ± 0.03 a	1.000	0.264	**0.008**
EC (dS/m)	0.15 ± 0.00 a	0.16 ± 0.00 b	**0.031**	0.368	**0.0008**
NO_3^-(mg/kg)	3.67 ± 0.46 a	2.29 ± 0.57 b	**0.023**	0.096	0.194
NH_4^+(mg/kg)	4.24 ± 0.30 a	5.90 ± 0.42 b	**0.001**	-0.019	0.565
P Olsen (mg/kg)	13.95 ± 1.62 a	14.97 ± 2.29 a	0.660	0.186	**0.009**
K^+ (mg/kg)	153.53 ± 21.48 a	381.00 ± 30.38 b	**0.023**	0.264	**0.005**
Ca^{2+} (mg/kg)	6,591.00 ± 105.9 a	6,451.00 ± 149.8 b	0.361	0.232	**0.002**
Mg^{2+} (mg/kg)	149.53 ± 35.53 a	342.00 ± 50.25 b	**0.001**	0.239	**0.020**
Ca^{2+}/Mg^{2+}	46.44 ± 3.59 a	26.42 ± 5.07 b	**0.000**	0.371	**0.0001**
Mg^{2+}/K^+	1.26 ± 0.21 a	1.55 ± 0.29 a	0.322	0.297	**0.002**
Ca^{2+}/K^+	57.1 ± 6.82 a	33.00 ± 9.64 b	**0.020**	0.209	**0.002**
Cu (mg/kg)	19.40 ± 2.070 a	12.50 ± 2.93 b	**0.026**	0.092	0.134
Fe (mg/kg)	74.50 ± 5.24 a	35.10 ± 7.41 b	**0.000**	0.121	0.114
SO_4(mg/kg)	22.13 ± 5.43 a	37.20 ± 7.69 a	0.062	0.204	**0.038**
Na (mg/kg)	16.30 ± 1.66 a	23.10 ± 2.35 b	**0.008**	0.140	0.111
Zn (mg/kg)	3.47 ± 0.30 a	2.47 ± 0.39 b	**0.016**	0.045	0.270
Clay (%)	17.50 ± 1.42 a	24.10 ± 2.01 b	**0.003**	0.405	**0.0001**
Silt (%)	36.50 ± 2.54 a	41.90 ± 3.60 a	0.140	0.405	**0.0001**
Sand (%)	45.90 ± 3.72 a	34.00 ± 5.26 b	**0.032**	0.353	**0.0002**

CONV vineyards showed higher values ($p< 0.05$) of sand, OOM at -20/-40cm, NO_3^-, Cu, Fe and Zn compared with ECO vineyards (Table 1). Interestingly, ECO management were related to a significant increase ($p <0.05$) of: Electrical Conductivity (EC), which could be explained by a higher concentration of Na^+, K^+ and Mg^{2+}, and N-NH_4^+, which could be derived from cow manure-based compost amended in the soil. ECO vineyards showed a higher level ($p<0.05$) of SOC (-20/40cm), ratio SOC (5-20cm/20-40cm), ratio OOM (5-20cm/20-

40cm), and the difference of OOM and SOC in the topsoil vs subsurface (Table 1). The results indicate that OOM could be highly degraded in the subsurface in ECO probably due to the presence of a more active soil microbiota. Mantel Test revealed, in order of relevance: Clay (texture), EC, Silt, K^+, Mg^{2+}, Ca/Mg, Ca/K, SOC and OOM ratios (topsoil/subsurface), OOM Diff (topsoil-subsurface) and OOM as main drivers to change the microbial community diversity. Interestingly, some significative soil parameters ECO vs CONV (p>0.05) did not show significative correlation (Mantel Test) with microbial community changes (pH, NO_3^-, NH_4^+, Cu, Fe, Na^+ and Zn), probably due to high dispersion among replicates in each plot.

Microbial diversity and Richness

Regarding quantitative effect of the management on microbial populations abundance (qPCR assessment), ECO management decreased significantly total bacterial populations (ECO: 2.1 · 10^9 gene copies · mL^{-1}, CONV. 3.5 · 10^9 copies g^{-1}; P=0.002, mainly when Macabeu variety was used; P>0,001). In addition, total bacteria under CONV treatment were significantly different in both varieties (P= 0.006). No significant effects of management (ECO and CONV) and variety were revealed for total fungi (3.8-2.7·10^8 ITS · g^{-1}; P=0.08), ammonium oxidizing bacteria (AOB) (1.9-0.8·10^8 *amoA* · g^{-1}; P=0.068) nor ammonium oxidizing archaea (AOA) (2.2-1.5 · 10^5 *amoA* · g^{-1}; P = 0,070). Quantitative results confirmed the high abundance of bacteria and fungi in the subsoil zone and the low prevalence of AOA. Interestingly, AOA abundance in our subsoil samples were low (<0,01%), with a high abundance of the AOB (2-9%), which was opposite to the high AOA abundance (1-10%) reported in (Gobbi et al., 2022).

A total of 2,972 ASVs were identified among the samples (n=29), with 15,058±6,124 reads/sample for CONV and 15,692± 3,051 reads/sample in ECO management. To perform alpha and beta diversity assessment rarefied samples were utilized for downstream analysis. ECO increased the inverted Simpson index (1/D; ECO: 6049; CONV: 470,1; P_{MW}= 0.017) but no effects were observed on the Shannon index (H) (ECO 6.94 ± 0.32; CONV: 6.93 ± 0.06; P_{MW}= 0.064). Richness index was not significantly affected by the type of the management (Chao 1: 2340; P_{MW} =0.647). Regarding the taxa distribution, a total of 13 phyla and 34 classes (Figure 1) above 0,1% of relative abundance (RA) have been identified. Dominant phyla above 5% of RA were in CON-ECO: *Proteobacteria* (31-34%), *Actinobacteria* (34-30%), *Acidobacteria* (14-15%), *Gemmatimonadetes* (6-4%), *Bacteroidetes* (5-6%), being only 4 Phyla differentially abundant: Proteobacteria (FDR=0.010), Actinobacteria (FDR=0.034) *Gemmatimonadetes* (FDR=0.031) and *Firmicutes* (FDR=0.031). ECO management increased *Proteobacteria*, whereas CONV increased the RA of *Actinobacteria*, *Gemmatimonadetes* and *Firmicutes* (FDR <0.05). Organomineral fertilizer utilization in CONV and the annual pruning wood incorporation to the soil both in ECO and CONV management could contribute to maintain the high abundance and microbial diversity, as well as a similar composition at phylum level.

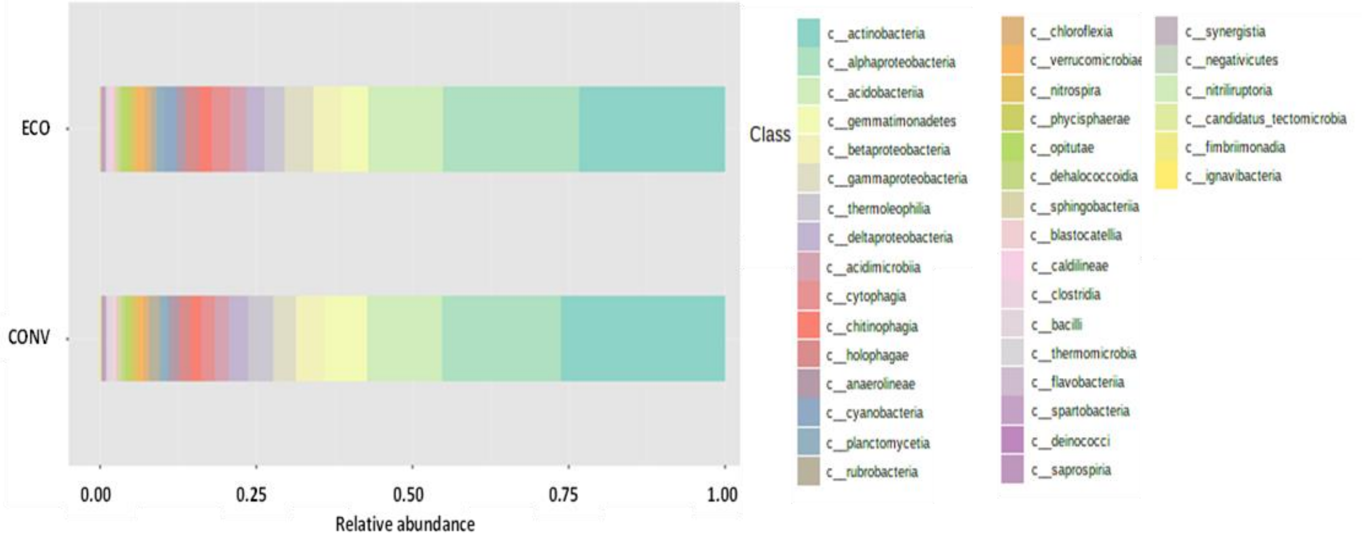

Figure 1. Microbial community distribution in vineyard soil (-20/-40cm) at Class level for each management strategy: ECO (n=14) and CONV (n=15). Classes >1% of relative abundance are reported.

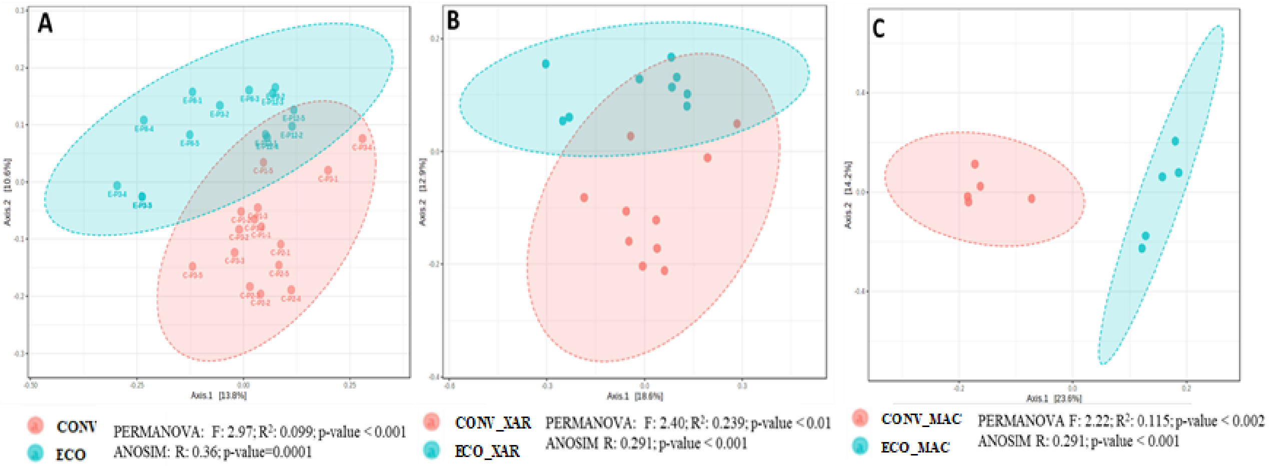

Figure 2. Principal coordinate analysis (PCoA) of bacterial communities (-20/-40cm soil) A) management strategy , B) management strategy in Xarel·lo vineyards, and C) the management strategy in Macabeu vineyards (C).

The main classes depicted were *Actinobacteria* (26-23%), *α-Proteobacteria* (19-22%), *Acidobacteria* (12%) of RA, followed by *Gemmatimonadetes*, *γ-Proteobacteria*, *Thermophilia*, *δ-Proteobacteria* and *Acidimicrobiia*, *Cytophagia*, and *Chitinophagia* above 2% of RA. Among them only *Gemmatimonadetes* (FDR=0.007), γ-Proteobacteria (FDR=0.019) and *Thermoleophilia* (FDR=0.031) were different between the managements. The most predominant genera and shared individual ASVs above 0,3% of RA were *Arthrobacter-Pseudoarthrobacter* (2 ASVs), *Dongia*, *Bradyrhizobium*, *Microcoleus* (Cyanobacteria), *Skermanella* (3 ASVs), *Sphingomonas*, *Acidobacterium* (2 ASVs Acidobateria GP4), and *Gemmatimonas* that highly contributed to the core microbiome. Regarding the beta diversity, PCoA with dissimilarity Bray Curtis distance index revealed that the management (ECO-CONV) contribute to differentiate the microbial community at ASV level (Fig. 2). ANOSIM and PERMANOVA assays confirmed the significance of the management effect on the microbial community structure (ANOSIM R:0,36 P=0.0001; PERMANOVA F:2,97 R2: 0,099 P<0,001) (Fig. 2). Vine variety did not influence on the microbial community structure in each management treatment (varieties in ECO ANOSIM, R: -0.0454, P: 0.5687; varieties in CONV ANOSIM, R: 0.1047, P: 0.2114). However, on an individual variety the management had a significant impact on microbiota (Xarel·lo ANOSIM, R: 0.291 P<0.001; Macabeu ANOSIM, R: 0,291, P<0,001).

Conclusion

Ecological management of vineyards has been confirmed to affect the microbial diversity in subsoil zone in the vineyards. The texture EC, and organic carbon at different depths, have been identified as the main driving environmental parameters that modulates the microbial community in vineyard subsurface soils. A combining impact of plot heterogeneity effect and the management has been identified in the present study. The results revealed the importance of combining a deep physicochemical characterization of numerous replicates with the microbial diversity data to gain better insights on the final contribution of the management (ECO vs CON), and to identify key soil and microbial parameters that impact into the soil microbiome of vineyards.

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