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Bacterial community dynamics varies with soil management and irrigation practices in grapevines (*Vitis vinifera* L.)

Stefanie N. Vink [a,*], Antonios Chrysargyris [b], Nikolaos Tzortzakis [b], Joana Falcão Salles [a]

[a] Groningen Institute for Evolutionary Life Sciences, University of Groningen, the Netherlands
[b] Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Limassol, Cyprus

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ABSTRACT

Plant-associated bacterial communities are important contributors towards plant health and productivity, and are increasingly acknowledged for having the potential to alter plants' response to climate extremes, such as drought. Viticulture is generally conducted in areas that are prone to seasonal water deficit, and irrigation is the common way to counter this. In addition, soil conservation measures, such as cover cropping and no-tillage, are increasingly used. However, the effect of these management practices, in particular in viticulture, on soil bacterial communities remains unclear. Therefore, the objective was to examine the effect of irrigation and tillage on shaping soil bacterial communities associated with different grapevine cultivars, at different plant phenological stages.

We conducted an experiment whereby we examined the soil bacterial community of three *Vitis vinifera* L. (grapevine) cultivars (two indigenous and one international, grown at different locations in the same vineyard) under different management practices (irrigated and non-irrigated, tillage and no-tillage). Since there is evidence that plant phenology can also affect soil microbial communities, we sampled soil at three different plant developmental stages (flowering, veraison and harvest).

Soil bacterial communities primarily correlated with cultivar and location. At harvest we observed a link between bacterial communities and grape quality and yield measures and at all stages with soil physicochemical properties. A number of taxa were enriched in one or two cultivars, but these were generally in low abundance in our dataset. We also found an effect of plant phenology and management practices within each cultivar. For the two indigenous cultivars, soil bacterial communities at the flowering stage were most distinct, while for the international cultivar it was at the veraison stage. However, for all three cultivars, this effect differed by tillage treatment: bacterial communities in the tillage treatment varied by plant phenology, but in the no-tillage treatment less difference was observed, suggesting a buffering effect. While irrigation did lead to changes in bacterial communities, the effect was not as strong as that of the other factors.

Our results show that soil bacterial communities in grapevines are shaped by both plant-associated effects (i.e. cultivar and plant phenological stage) and environmental variables (i.e. soil management and soil physicochemical properties), and importantly, that these factors have an interactive effect. Future research on soil bacterial communities in grapevines should take this variability into account, as well as focusing on its functional implications.

1. Introduction

Viticulture (grape cultivation) is one of the oldest know agricultural systems. Globally, approximately 7.4 million ha of agricultural land was used for viticulture in 2018, primarily used for the production of wine, but also for table grapes and raisins (OIV, 2019). Common agricultural practice in viticulture is a combination of tillage and irrigation several times a year. However, these practices are known to disturb the soil

Abbreviations: T, tillage; NT, no-tillage; I, irrigated; NI, non-irrigated; EC, electrical conductivity; OM, organic matter; TSS, total soluble solids; TA, titratable acidity; AA, ascorbic acid; SVs, sequence variants; PCoA, Principal coordinate analysis; PERMANOVA, permutational multivariate anova; CAP, constrained analysis of principal coordinates; PCA, principal component analysis.

* Corresponding author.
E-mail address: s.n.vink@rug.nl (S.N. Vink).

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ecosystem - affecting it both in terms of soil characteristics and soil microbial functioning (Hartman et al., 2018). This can indirectly affect many different facets of plant health, such as disease incidence and access to nutrients and water. Particularly important in an agricultural setting is that a diverse cover crop could lead to a decrease in pathogen loads (Guzmán et al., 2019; Vukicevish et al., 2017). For many wine growing regions, climate change is predicted to lead to an increase in water deficit, requiring large adaptations to maintain production (Santillán et al., 2019).

To counter these issues, different management practices have been proposed and are increasingly employed. This includes the use of drought-resistant cultivars (Fraga et al., 2013) as well as different soil conservation measures, such as cover cropping and no-tillage. No-till management often includes a naturally developed vegetation (Guzmán et al., 2019) and can lead to reduced soil erosion, increased soil organic matter content and improved soil aggregate stability and water-holding capacity (Six and Paustian, 2014; Triplett and Dick, 2008), which could greatly benefit vineyards in periods of drought.

Plant-associated soil microorganisms can offer substantial benefits to plants and are vital to plant health and productivity. This occurs both directly, through the actions of mutualists and pathogens, but also indirectly through changes in biogeochemical cycling. Bacterial communities, as well as individual bacterial species, can alter a wide array of plant functional traits (Goh et al., 2013), including specific leaf area, nutrient uptake strategies and drought resistance (Friesen et al., 2011; Rolli et al., 2015) and resilience and resistance towards pathogen invasions (Wei et al., 2015). Rhizosphere microbial communities are selected primarily through specific root exudates produced by the plant (Hartmann et al., 2009; Mendes et al., 2013) and can vary greatly between plant species (Berg and Smalla, 2009), but also at the cultivar level in a number of plant species (Jiang et al., 2017; Liu et al., 2019; Mendes et al., 2017), including grapevines (Berlanas et al., 2019; Marasco et al., 2018). Furthermore, the interaction between plants and their microbiomes can vary both over time, and with different plant phenological stages (Wagner et al., 2016) as a result of changing root exudation patterns (Chaparro et al., 2014).

Many studies have been conducted on the effect of different viticulture management practices on grapevine health, but less is known about how the microbial communities associated with the plants and soil are affected. Likar et al. (2017) showed that soil microbial diversity varied considerably between vineyards under conventional and ecological management, whereby tillage in particular has a strong effect on bacterial communities. Similarly, distinct bacterial communities were found with different agricultural practices (Vega-Avila et al., 2015) and soil management, more than seasonal and phenological changes, affected the abundance of culturable microorganisms (López-Piñeiro et al., 2013).

While it is clear that both environmental, i.e. edaphics and management practices, and plant derived factors are important in shaping bacterial communities, the combined effects have not been studied well in viticulture. The objective of this study was therefore 1) to examine how irrigation and tillage shape bacterial communities associated with different grapevine cultivars at different plant phenological stages and 2) to ascertain whether differences existed between cultivars, both in terms of these processes and in specific members of their bacterial communities, and whether we could link changes to grape quality and yield.

2. Materials and methods

2.1. Site description

The experiment was conducted in 2017 at the Mallia winery commercial vineyards (34°48′58.1"N 32°47′07.0″E, 645 m) in Limassol, Cyprus. The vineyard has a dry microclimate of less than 30 mm of summer rainfall from June to August, with 30.0 °C average midday temperature and 30% air humidity during the summer months of on-vine ripening. Three grapevine cultivars Chardonnay, Maratheftiko and Xynisteri (all *Vitis vinifera* L.) were used at locations within 1 km of each other within the vineyard. Soils were calcaric leptic cambisols (FAO, 2014) with a soil texture of clay loam (Maratheftiko) or clay (Chardonnay and Xynisteri). Chardonnay occupied approximately 0.7 ha, Maratheftiko approximately 0.6 ha, and Xynisteri approximately 2.6 ha.

Chardonnay is a globally-used white grape variety and sensitive to drought stress (Hatmi et al., 2015). Xynisteri (white grape) and Maratheftiko (red grape) are both indigenous to Cyprus and known to be tolerant to drought stress (Chrysargyris et al., 2018; Tzortzakis et al., 2020). All three cultivars were between 10 and 15 years old and own rooted. Vines were trained using a traditional bilateral 'royat' system with spacing of 1.5 m in north–south-orientated rows, 2.4 m between the rows, and a plant density of 2700 plants ha^{-1}.

2.2. Experimental design and treatments

For each cultivar there was a split-block design: in March 2017 each area was divided into four treatment subplots which were further divided into four replicate plots consisting of 5 vines. The treatments were tillage (T) and no-tillage (NT) crossed with irrigated (I) and non-irrigated (NI). The T treatment was bare soil, with tillage taking place twice through the examined growing period while the NT treatment consisted of naturally growing vegetation. Irrigation took place with a drip irrigation system at four time points throughout the growing season, starting at the end of May, after the first sampling point, to a volumetric water content of 20–30% as described previously (Chrysargyris et al., 2018).

During the experiment common cultivation practices of fertilizer (20-10-10 N-P-K once a year and 0-0-30 every 3–4 years) and pesticide (sulfur and insecticides 3–4 times per year) applications were followed.

2.3. Soil sampling, soil physicochemical properties and yield and grape quality

Soil samples were collected at three different plant phenological stages: flowering, veraison (when the grapes start ripening) and harvest. For Chardonnay, this corresponded to mid-May, end-July and end-August respectively, while for Maratheftiko and Xynisteri this was mid-May, end-August and end-September respectively. Only NI soil samples were collected at the first sampling point, as at this time point the irrigation treatment had not yet been initiated. For the following two sampling points the full 2 water × 2 soil management treatments were sampled, resulting in a total of 132 samples.

Soil samples were collected in the vicinity of plant roots from each replicate plot (of 5 plants) within each treatment at each sampling date by taking four soil cores (top-layer, 15–30 cm) with a clean corer (50 mm diameter) or hoe, both washed and disinfected with 70% ethanol between sampling, and combining to make a single heterogeneous composite sample (approximately 3–4 kg). Soil samples were placed inside marked bags and transported to the laboratory in cooler boxes for storage at 4 °C and −20 °C and processed within several days for measurement of physicochemical properties and DNA extraction, respectively.

Soil physicochemical properties were measured on the same biological samples used for bacterial community analysis using methods previously described (Carter and Gregorich, 2008; Stamatakis and Chrysargyris, 2017). Soil was air-dried and hand-sieved to pass a 2 mm mesh. Equivalent calcium carbonate ($CaCO_3$) was determined using the calcimeter method. Electrical conductivity (EC) and pH were determined according to 1:1 soil to solution ratio, employing a portable pH/EC-meter (HI 98130 HR, Hanna Instruments, USA). Soil potassium (K) and sodium (Na) were extracted with neutral ammonium acetate and were measured with a flame photometer (JENWAY, PEP-7 Jenway,

Dunmow, UK), and total nitrogen (N) was determined by means of Kjeldahl (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Oldham, UK). Soil type (percentage of sand, silt and clay) was observed by hydrometer method with Bouyoucos scale and organic matter content was determined with the Walkley-Black volumetric method. Mean values per cultivar and plant phenological stage are presented in Table S1.

At the final sampling time, yield and grape quality was evaluated for all replicates (from 2 plants per plot) per treatment and cultivar. Yield was measured as grape weight (kg per plant), mean cluster fresh weight (g) and number of clusters per plant. Grape quality was assessed by measuring total soluble solids (TSS; expressed in % sugars), pH, titratable acidity (TA; expressed in % tartaric acid), ascorbic acid (AA; expressed in mg 100 mL^{-1} grape juice), and total grape phenol (gallic acid equivalent - GAE 100 g^{-1} fresh weight), anthocyanin (mg cyn-3-glu 100 g^{-1} fresh weight) and tannins (mg 100 mL^{-1} grape juice). Methods were as in Chrysargyris et al. (2018).

2.4. DNA extraction, sequencing and sequence data processing

DNA was extracted using the PowerSoil DNA extraction kit (Qiagen) following the manufacturer's instructions on a subsample of 0.5 g of soil. Approximately 50 mg of sterile sand (ø 0.1 mm, BioSpec Products, Bartlesville, USA) was added to improve yield. DNA was purified, pooled (with normalization based on 16S quantitative PCR) and sequenced at the University of Minnesota Genomics Center using a 2 × 250 bp MiSeq run (Illumina) on the V4 region of the 16S rRNA (515F–806R primer pair; Caporaso et al., 2011).

Sequences were demultiplexed at the sequencing center and subsequently quality filtered using the QIIME2 v 2018.6 platform (Bolyen et al., 2018). Briefly, primer sequences were removed using the cutadapt plugin (Martin, 2011), and the DADA2 plugin (Callahan et al., 2016) was used to filter, infer sequence variants, identify chimeras, and merge paired-end reads. The resulting representative sequence variants (SVs) were aligned using MAFFT (Katoh and Standley, 2013) and used to generate a mid-point rooted phylogenetic tree in Fasttree (Price et al., 2010). Taxonomic assignments were conducted using the RDP classifier v.2.10 (Wang et al., 2007). Full details and setting are presented in the supplementary materials. Forward and reverse sequencing reads without primer sequences were submitted to the European Nucleotide Archive under accession nr. PRJEB40549 (available at https://www.ebi.ac.uk/ena/data/view/PRJEB40549).

2.5. Statistical analysis

All statistical analyses were conducted in R version 3.5.1 (R Core Team, 2018). Prior to statistical analysis, non-bacterial and sequences of chloroplast and mitochondrial origin were removed. Samples were rarefied to 13,976 sequences per sample to take any differences in sequencing depth into account. Because we were interested in both differences between cultivars and within each cultivar, we used the full data set and data subsets from each cultivar.

Observed SVs, Shannon diversity and Simpson measurements were calculated using the package Phyloseq (McMurdie and Holmes, 2013), and Faith's phylogenetic distance was calculated using the packages picante (Kembel et al., 2010) and btools (Battaglia, 2020). ANOVA was conducted on each cultivar dataset to determine the effect of plant phenology and treatment on alpha diversity.

Differences in bacterial β-diversity were examined using the package vegan v. 2.5–3 (Oksanen et al., 2018) in R. Principal coordinate analysis (PCoA) using Bray-Curtis dissimilarities was used to visualize differences in bacterial communities between cultivars and, within each cultivar, between different plant phenological stages and treatments. Significant differences were tested using permutational multivariate anova (PERMANOVA, Anderson et al., 2006) with 999 permutations, using the function *adonis*. Since PERMANOVA is sensitive to differences in dispersion between groups, function *betadisper* (and *permutest.betadisper*, using 999 permutations) was used to assess variability in bacterial community structure between cultivars. To determine bacterial turnover per cultivar and treatment we calculated all pair-wise Bray-Curtis dissimilarities within each cultivar using the function *distance* in Phyloseq, and retained only those between consecutive plant phenological stages for each treatment replicate. Significant differences between treatment cultivar and plant phenological stage contrast were tested using anova with non-significant model terms removed. Constrained analysis of principal coordinates (CAP) was used to determine possible associations between bacterial community structure and soil physicochemical and plant measurements using function *capscale*. Since different measures were taken at different plant phenological stages, we conducted two separate analyses; one using soil physicochemical properties at all stages, and one using grape yield and quality data at harvest. Function *anova.cca* (using 999 permutations) was used to determine the significance of each measurement and axis of the capscale model. To determine whether differences existed in terms of soil physicochemical measurements between cultivar locations principal component analysis (PCA) was conducted using vegan on all measures except soil type. Prior to analysis data were scaled to zero mean and unit variance.

Taxonomy at the phylum level was visualized using Phyloseq for each cultivar and treatment combination. Quantitative differences in genera counts between different cultivars were assessed with package DESEQ2 in R (Love et al., 2014), using SVs that were merged to genera (and abundances summed) and subsequently filtered to remove those with a total abundance of <100 and occurrence in less than 5% of all samples. Any genera that did not differ significantly in at least one pairwise comparison were removed from the final dataset. Mean abundance per cultivar of each genus in the DESEQ2 dataset was calculated in Phyloseq to compare quantitative differences to abundances.

3. Results

3.1. Effect of plant phenology and treatment on alpha-diversity within each cultivar

Following quality filtering and rarefaction, a total number of 1,872,784 reads distributed over 30,498 SVs remained in the dataset, with between 708 and 2881 SVs per sample. Of these SVs, the vast majority belonged to the rare fraction - 99.5% of all SVs had a relative abundance of 0.1% or less.

Observed richness of SVs varied according to plant phenological stage, treatment and cultivar (Fig. 1a, b & c). In all three cultivars, the treatments showed an increase in observed SV richness at harvest, but the picture was more complex for the tillage treatments at harvest and all treatments at veraison. For the Chardonnay samples, observed richness differed significantly over the three phenological stages (ANOVA: $F = 15.7, p < 0.001$), between the different treatments (ANOVA: $F = 3.3, p < 0.05$) and their interaction (ANOVA: $F = 4.2, p < 0.001$) (Fig. 1a). Overall, richness increased at harvest, with the most pronounced effect in the NI-NT treatment. The changes in richness over time showed a similar pattern for all treatments, with an initial small decrease at veraison, followed by a small increase at harvest (Fig. 1d). An exception to this pattern was the I-NT treatment, which did not see an initial decline. In the Maratheftiko samples, the different treatments did not specifically lead to a change in richness (ANOVA: $F = 1.4$, $p =$ n.s.), but there was a significant effect of plant phenology (ANOVA: $F = 4.3, p < 0.05$) and the interaction between the two (ANOVA: $F = 6.2, p < 0.01$) (Fig. 1b). Changes in observed richness relative to flowering showed a differentiation between the two soil treatments, with the T samples showing a decrease while the NT samples increased in richness over time (Fig. 1e). Differences in observed richness was marginally significant between the different phenological stages (ANOVA: $F = 5.4, p < 0.05$), treatments (ANOVA: $F = 3.3, p < 0.05$) and their interaction (ANOVA: F

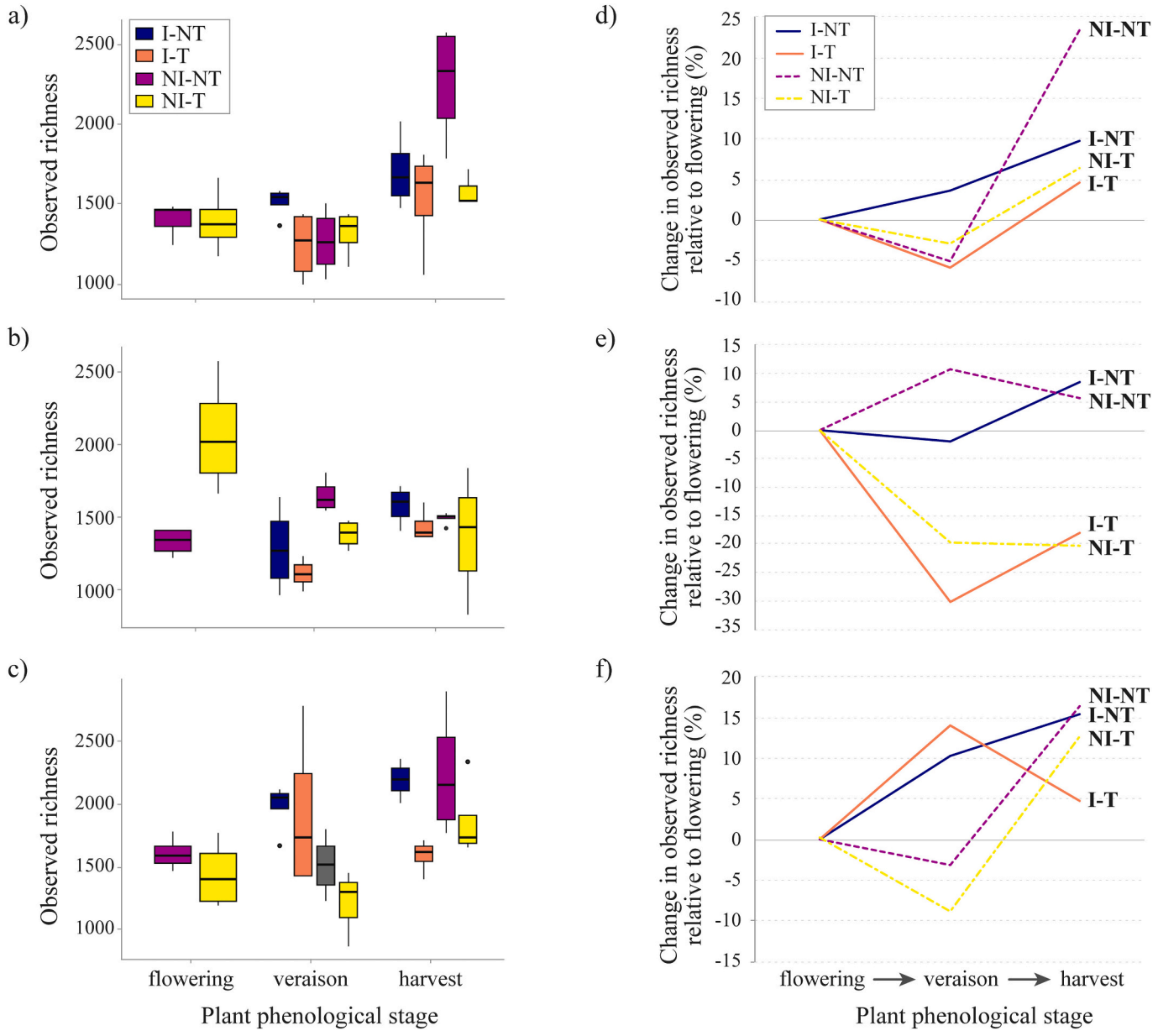

Fig. 1. Observed richness of sequence variants per cultivar, plant phenological stage and treatment. The left panels show the observed richness values (median, quartiles) of a) Chardonnay, b), Maratheftiko and c) Xynisteri. The right panel show the change in observed richness (in %) relative to the value at flowering (before irrigation) for d) Chardonnay, e) Maratheftiko and f) Xynisteri. Treatments were as follows; I-NT = irrigated, no-tillage, I-T = irrigated, tillage, NI-NT = non-irrigated, no-tillage, NI-T = non-irrigated, tillage. At flowering only the non-irrigated plots were sampled.(For a colour figure, the reader if referred to the web version of this article)

= 3.1, $p < 0.05$) in the Xynisteri samples. Irrigation initially resulted in an increase in observed richness, irrespective of soil management, but at the final sampling time richness increased for all treatment, with the exception of the I-T treatment (Fig. 1c & f). Similar patterns to those of the observed SV richness were seen for Shannon and Simpsons indices and Faith's Phylogenetic distance (data not shown).

3.2. Beta-diversity and bacterial turnover

A significant difference in the bacterial communities associated with each cultivar (PERMANOVA: $R^2 = 0.1$, $p < 0.001$) was observed, with the main separation between Chardonnay and Maratheftiko, and Xynisteri in between the two (Fig. S1) and this factor had the largest effect size of all factors examined (Table S2). Although the Chardonnay and Xynisteri samples were more variable in their bacterial community composition than those of Maratheftiko, this difference was not significant (beta dispersion test: $F = 0.19$, $p = 0.833$).

Within each cultivar, the effect of the different treatments and plant phenological stage differed significantly (Table 1, Fig. 2a & b). For all three cultivars, plant development stage and the interaction between plant phenology and soil management accounted for the highest amount of variability in bacterial community structure, while water management accounted for the least amount of variability. This was apparent in the PCoA plots, where there was a clear differentiation, particularly in the T treatments. This was also reflected in turnover, where the NT-T treatments across all cultivars showed a high turnover (mean Bray Curtis dissimilarity = 0.62 vs 0.58, 0.55 and 0.57 for I-T, I-NT and NI-NT resp.; $F = 8.30$, $p < 0.001$), although this varied per cultivar (treatment: cultivar interaction, $F = 4.44$, $p < 0.001$). In addition, turnover of bacterial communities was dependent on plant phenology; it was

Table 1
PERMANOVA results per cultivar for samples at different plant phenological stages (flowering, veraison and harvest), soil management (no-tillage or tillage), water management (irrigated or non-irrigated) and their interactions.

	Chardonnay			Maratheftiko			Xynisteri		
	F	R^2	*p*	*F*	R^2	*p*	*F*	R^2	*p*
Plant phenology (PP)	2.87	0.12	0.001	1.78	0.09	0.001	1.50	0.07	0.002
Soil management (SM)	2.78	0.06	0.001	2.30	0.06	0.001	2.18	0.05	0.001
Water management (WM)	2.36	0.05	0.002	1.26	0.03	0.033	1.30	0.03	0.027
PP:SM	2.19	0.09	0.001	1.20	0.06	0.021	1.53	0.07	0.001
PP:WM	1.50	0.03	0.032	1.32	0.03	0.012	1.14	0.03	0.109
SM:WM	1.25	0.03	0.093	1.12	0.03	0.133	1.30	0.03	0.028
PP:SM:WM	1.17	0.02	0.161	1.01	0.03	0.408	1.07	0.03	0.207

significantly higher between flowering and veraison (mean Bray Curtis dissimilarity = 0.61) than between veraison and harvest (mean Bray Curtis dissimilarity = 0.57) for all cultivars ($F = 9.21$, $p = 0.004$).

In the Chardonnay samples the veraison samples separated from the flowering and harvest samples in the first axis, while the flowering and veraison samples differed in axis 2. At both flowering and veraison, NT and T samples differed considerably in their bacterial communities, while at harvest it was more nuanced with a strong differentiation between I-NT and NI-T samples. In terms of bacterial community turnover, community composition changed more from flowering to veraison, than from veraison to harvest in the NT treatments, but this was not the case for the treatments, where turnover remained high (Fig. 3 left panel). Bacterial communities from the I and NI treatments differed significantly for both main and interaction terms (Table 1), although these differences did not exhibit a clear pattern. In the Maratheftiko samples a similar pattern as for Chardonnay emerged, although it differed somewhat (Fig. 2c & d). Different communities were apparent, but here it occurred between flowering and the other two phenological stages, but again this was most evident in the T treatment. The T and NT samples differed from each other at flowering, but where more similar at the latter stages, with considerable overlap. Again, irrigation did not appear to affect bacterial communities as much, which was reflected in the PERMANOVA output (Table 1) where it was significant (both as main effect and in interactions) at the $p < 0.05$ level only. Bacterial turnover was lower in the T samples than those with NT, and here too change in bacterial communities differed between flowering-veraison and veraison-harvest in the NT samples (Fig. 3 middle panel). However, this difference was lower than observed in the Chardonnay samples. Finally, in the Xynisteri samples, the same pattern in β-diversity as in Maratheftiko was observed, the bacterial communities at flowering were different to those at veraison and harvest, but only in the NT samples (Fig. 2d & e). Here too, bacterial communities differed between T and NT only at flowering and little effect of irrigation was observed. Turnover decreased from the first two stages to the final two stages, but for this cultivar this pattern was evident in both NT as T samples (Fig. 3 right panel). In fact, the decrease in turnover was largest in the I-T samples.

CAP analysis using the soil data also showed a clear separation to cultivar, with pH, EC, OM content, Na and K significantly affecting the model (Fig. 4a). pH (pseudo $F = 2.4$, $p = 0.001$) was negatively and EC (pseudo $F = 1.5$, $p = 0.033$), Na (pseudo $F = 1.7$, $p = 0.005$) and K (pseudo $F = 1.7$, $p = 0.012$) were positively related to the Maratheftiko. Soil OM content (pseudo $F = 4.1$, $p = 0.001$) was positively related with the Xynisteri samples and negatively with those of Chardonnay. Principal component analysis of soil physicochemical properties showed a clear separation between the Maratheftiko sites and those of Chardonnay and Xynisteri, but not between Chardonnay and Xynisteri (Fig. S2). The separation was driven by pH on the one hand, which was higher in the Chardonnay and Xynisteri sites, and higher levels of Na, N and K in the Maratheftiko sites.

While overall the model using the harvest data was highly significant (pseudo $F = 1.3$, $p = 0.001$), and here too there was a clear separation to cultivar, the relationship with bacterial communities was not as clear and only four of the measures were significantly correlated with bacterial community composition (Fig. 4b). In terms of yield, only the number of clusters was significantly correlated (pseudo $F = 1.4$, $p = 0.036$), with a negative relationship with the Chardonnay samples. Total grape acidity (pseudo $F = 1.6$, $p = 0.019$) was positively linked with Chardonnay samples, and anthocyanin levels (pseudo $F = 1.6$, $p = 0.023$) positively with Maratheftiko, while total soluble solids was negatively correlated with Xynisteri bacterial communities (pseudo $F = 1.8$, $p = 0.007$).

3.3. Taxonomic composition and variation per cultivar

At the phylum level samples were dominated by *Actinobacteria* (38% on average across all samples), *Proteobacteria* (30%) and *Acidobacteria* (12%) – typical for soils world-wide (Delgado-Baquerizo et al., 2018) - and overall there was little variation in these values between the different cultivars, plant phenological stages and treatments (Fig. S3).

While at a higher taxonomic level we did not observe differences, differential abundance analysis (DESEQ) showed significant differences between cultivars for various genera (Fig. 5a). However, of the 84 genera that were significantly higher in one or a combination of two cultivars, only a few had a clear higher occurrence in a single cultivar, while the majority of genera were either more prevalent in two cultivars or showed only a slight increase. The Chardonnay samples showed a strong differential abundance of the genera *Marihabitans*, *Propionispira* and *Rhodoligotrophos*. The genera that associated most strongly with Maratheftiko were *Jonquetella*, *Petrobacter*, and *Thiobacillus*. The Maratheftiko samples also were enriched with thermotolerant organisms, such as those from *Thermomonospora*. Finally, the genera Gp1, *Hydrogenophaga*, *Mucilaginibacter* and *Tumebacillus* showed a strong preference for the Xynisteri samples.

However, the genera that associated primarily with a single cultivar had a low overall abundance (Fig. 5b), while the genera with higher abundances, such as *Arthrobacter*, *Gaiella*, Gp16 and *Skermanella*, only showed a weak preference for one of the cultivars (or combination of cultivars). Four of the genera that showed a preference towards particular grapevine cultivars are known to contain members that have biocontrol and/or biofertilizing properties (Vacheron et al., 2013); *Arthrobacter*, with a high overall abundance, and *Bradyrhizobium* showed a slight preference for Chardonnay, while *Bacillus* was associated with samples of Maratheftiko and Xynisteri and *Rhizobium* with Xynisteri. However, none of these associations was particularly strong.

4. Discussion

We conducted this experiment to assess whether the grapevine soil microbiome was altered by different management practices (tillage vs no-tillage and irrigated vs non-irrigated) and plant phenology (flowering, veraison, harvesting) of three grapevine cultivars, and observed clear differences in bacterial communities for all of these.

The composition of the soil bacterial communities associated with

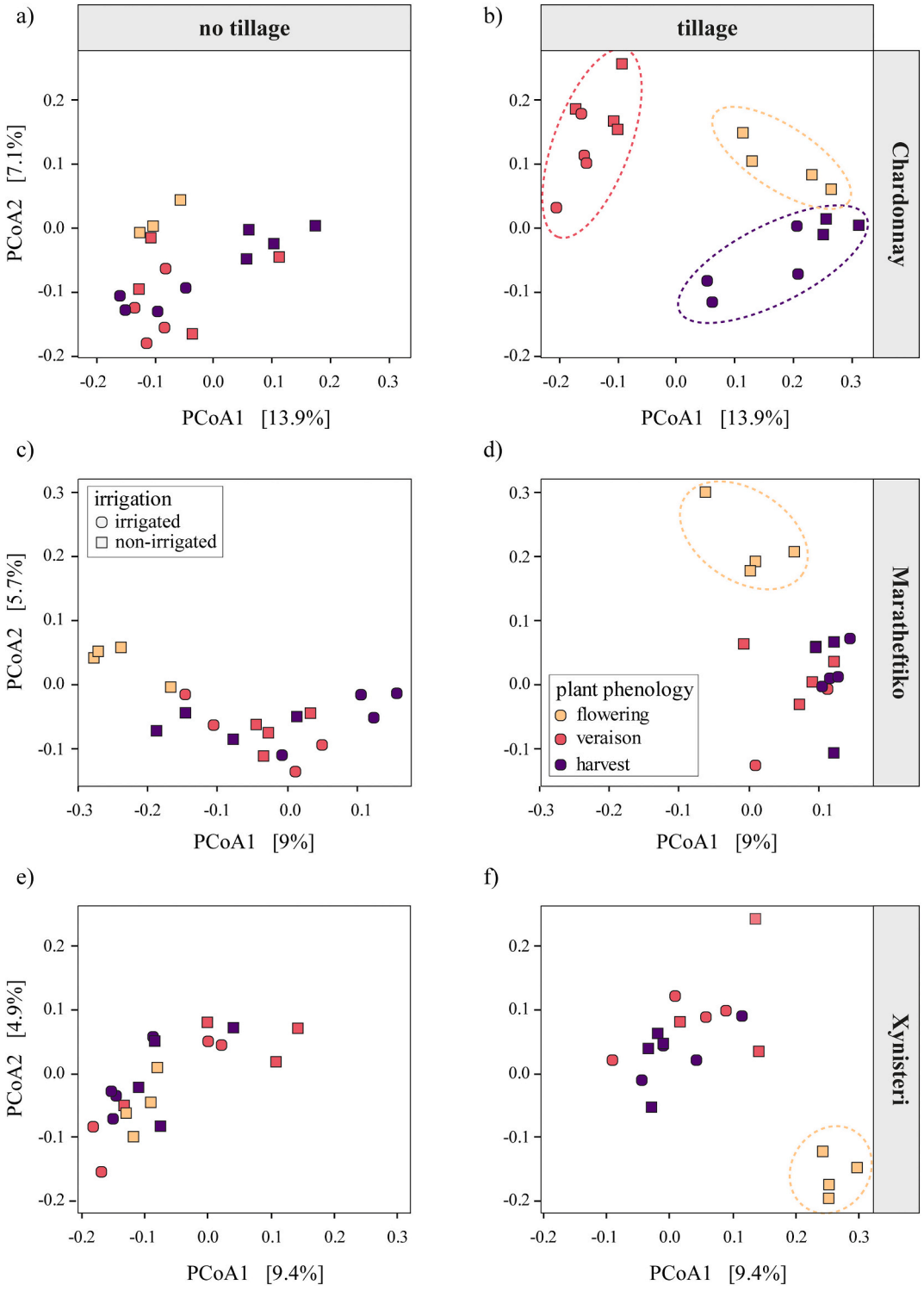

Fig. 2. Bacterial community structure using principal coordinate analysis of the Chardonnay (a and b), Maratheftiko (c and d) and Xynisteri (e and f) soil samples at different plant phenological stages (red = flowering, green = veraison and blue = harvest) separated per no tillage (a, c and e) and tillage (b, d and f), and per irrigated (circles) and non-irrigated (square) treatments. (For a colour figure, the reader if referred to the web version of this article)

the roots of the different grapevine cultivars showed the strongest differentiation between cultivars. While for α-diversity there were no obvious difference between cultivars overall, there was an interactive effect between plant phenotype and cultivar. But the most obvious differences were observed in terms of β-diversity, where the factor cultivar had the largest effect size and was therefore most able to explain variation in soil bacterial communities. CAP analysis identified that each community was linked to different soil physicochemicals and, at the final sampling point, to grape yield and quality. The bacterial community associated with the Maratheftiko cultivar in particular was correlated to many of the soil physicochemical properties we measured, and also showed a correlation with grape quality (but not yield) measures. This cultivar also presented a higher abundance of thermophilic genera and those capable of utilizing "alternative" energy sources, such as

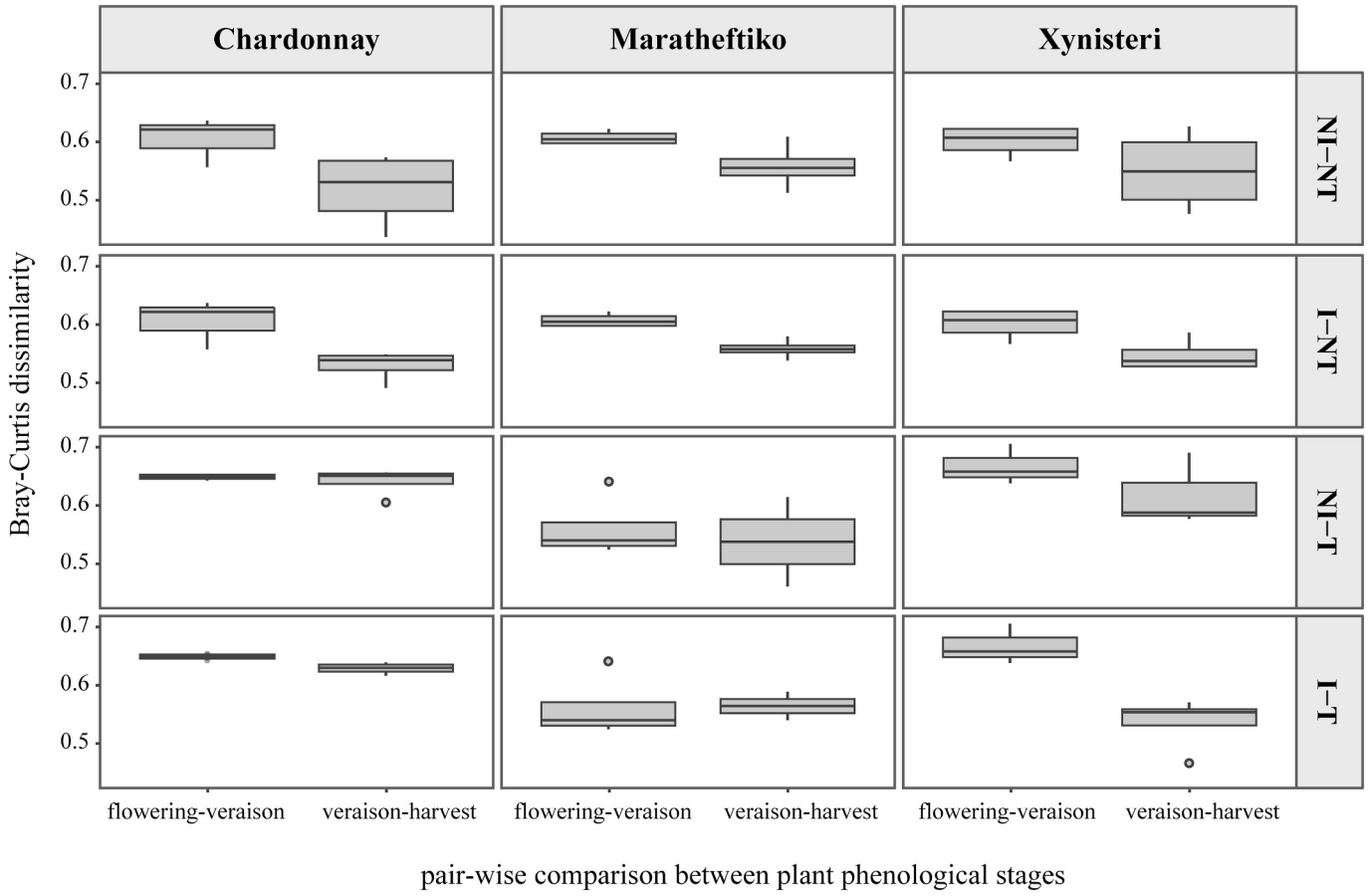

Fig. 3. Turnover of bacterial communities per cultivar and treatments over time. Turnover is represented as the Bray-Curtis dissimilarity of the pair-wise comparison between flowering to veraison and from veraison to harvest with higher values indicating that bacterial communities are more different to each other over the compared time points. Treatment codes are as follows: NI = non-irrigated, I = irrigated, NT = no-tillage, T = tillage.

heavy oil (Salinas et al., 2004) and sulfur (Tian et al., 2017). The other two cultivars were enriched with genera whose functioning was less clear, highlighting the lack of knowledge on both bacterial identity and functioning. Maratheftiko was the only red grape variety in our study, a potential reason for this separation, but since all three cultivars showed divergent communities, it seems likely that a more complex set of traits, inherent to cultivar identity played a role. Different cultivars of the same plant species can have profound effects on bacterial community composition (Jiang et al., 2017; Liu et al., 2019; Mendes et al., 2017), including in grapevines (Berlanas et al., 2019; Marasco et al., 2018). In addition, root exudates and morphology, in combination with soil factors, are known to exert a large influence on bacterial communities in soil (Berg and Smalla, 2009). These exudation patterns show considerable variability between different plant species and varieties (Inceoğlu et al., 2011) and can lead to distinct microbial populations (Jiang et al., 2017; Oger et al., 2004). Interestingly, we also did not observe specific differences in bacterial communities between the two indigenous cultivars, Maratheftiko and Xynisteri, and the international cultivar, even though Chardonnay is less drought tolerant and differs in terms of plant phenology, with grape ripening approximately a month earlier than the indigenous grape varieties. While indigenous grapevines are increasingly being studied for, amongst others, their ability to tolerate drought (Theodorou et al., 2019; Tzortzakis et al., 2020), to date much less is known about their effects on bacterial communities. Campisano et al. (2015) observed that different root endophyte bacteria associate with wild vs domesticated grapevine, but to our knowledge no studies have specifically addressed this in soils.

Besides differences in inherent plant properties, the differences we observed between cultivars could also be due to local biogeography, since the different cultivars were grown at different locations within the same vineyard resulting in confounding between location and cultivar. Edaphic variation at different geographical scales have been shown to be major determinants of soil and grape must microbial communities in vineyards (Bokulich et al., 2014; Burns et al., 2015; Zarraonaindia et al., 2015). Although there was a clear difference in soil physicochemical properties between the Maratheftiko site and the Chardonnay and Xynisteri sites (Fig. S2), there was much less differences between the latter two, despite clear differences in bacterial communities between all three (Fig. S1). It therefore seems likely that both biogeography and inherent plant properties combine to determine soil bacterial communities (Mezzasalma et al., 2018), but is remains important to better understand this variation since it can have functional implications in terms plant health, and, in the case of grapevines, for downstream wine quality (Gilbert et al., 2014; Zarraonaindia et al., 2015).

Differentiation between cultivars (and/or location) was apparent as the dominant factor in structuring bacterial communities, but it was not the only one, and we also observed trends in relation to plant phenology and management. In fact, for α- and β-diversity, and turnover (i.e. the change in bacterial community composition) there were clear differences based on plant phenology for all cultivars, but it was also highly dependent on soil management. In general, bacterial communities in the tillage treatments were more dissimilar than those in the no-tillage treatment. Tillage is known to have a large effect on bacterial communities (Hartman et al., 2018), and in vineyards it has been associated with reductions in soil carbon and nitrogen pools and aggregate stability (Belmonte et al., 2018; Pingel et al., 2019). In our experiment understory plants were allowed to develop naturally in the no-tillage treatment and thus these treatments were in effect a combination of no-

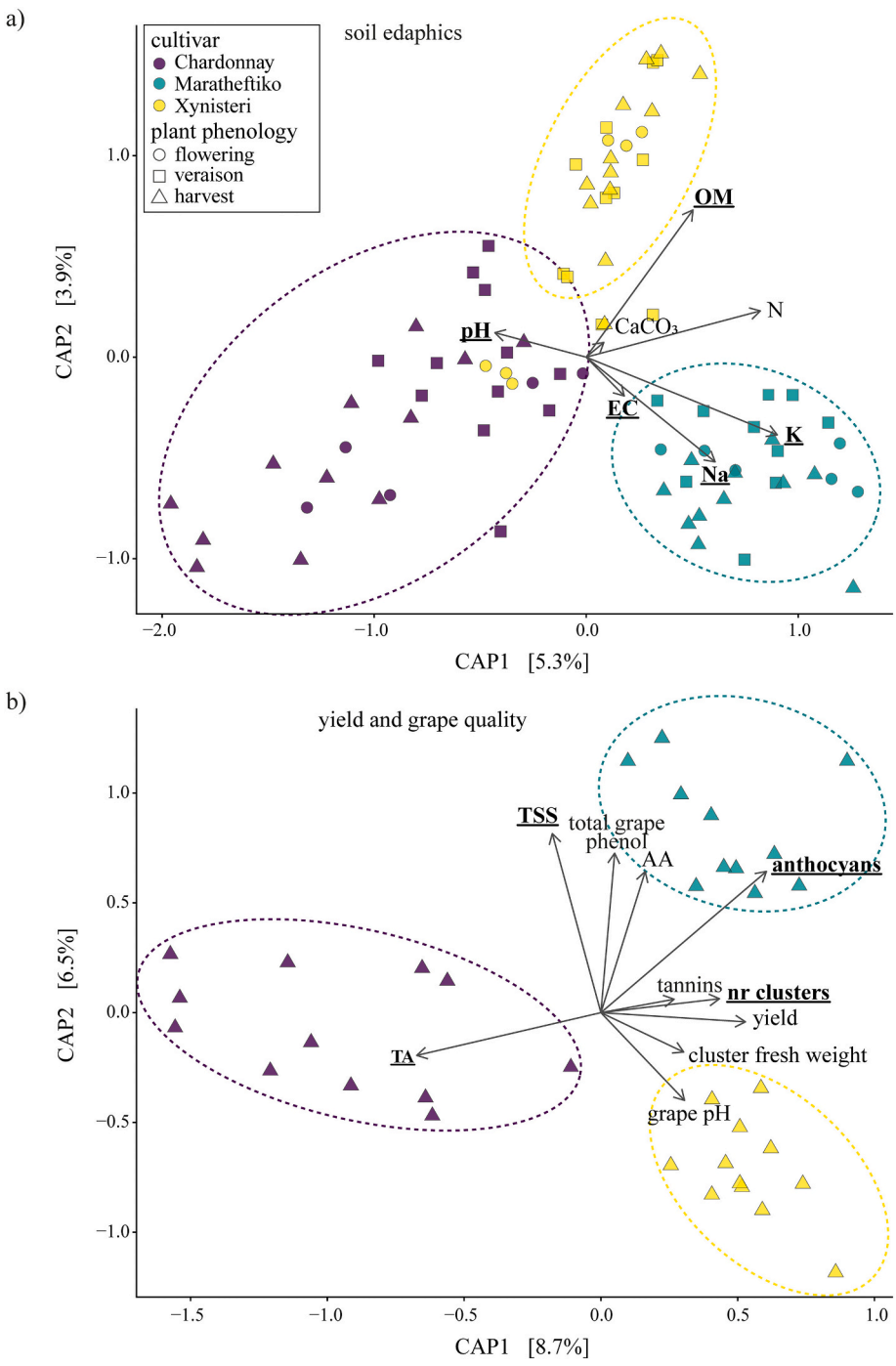

Fig. 4. Relationship between soil bacterial communities and soil physicochemical properties (a) and grape yield and quality (b) using constrained analysis of principal coordinates. For soil physicochemical the comparison include data from each plant phenological stage, while for the grape yield and quality data is only from harvest time point. Variables that were significantly correlated with soil bacterial communities are in bold and underlined. (For a colour figure, the reader if referred to the web version of this article)

tillage and cover cropping. Burns et al. (2016) studied bacterial communities in vineyards across California under different management practices and found that cover cropping had a larger influence in shaping soil bacterial communities than the absence of tillage did. However, from our results we cannot distinguish the two, but our samples showed less variation with respects to plant phenology in the no-tillage treatment than it did under tillage and we hypothesize that the buffering effect of this treatment could be due to the known improvement of soils measures such as functional microbial diversity, organic matter content, aggregate stability, or reduction in soil erosion in both cover cropping and no-tillage in vineyards (Belmonte et al., 2018; Capó-Bauçà et al., 2019; Goulet et al., 2004; Pingel et al., 2019; Ruiz-Colmenero et al., 2013).

The exudates produced by plant roots are known to differ depending on plant developmental stage, leading to distinct patterns of microbial community assembly (Chaparro et al., 2014). We observed a higher turnover of bacterial communities between flowering and veraison than between veraison and harvest under no-tillage soil management, and for Xynisteri, also for tillage. This, again, was somewhat dependent on soil management - for the Chardonnay and Xynisteri samples this was particularly true under tillage. Mobilization of N and C showed a distinct temporal pattern within grapevine root tissue (Zapata et al., 2004) and inter-annual variation was found to be a significant explanatory variable, but not the main one, in explaining bulksoil and root zone bacterial communities in a large study on Merlot-associated bacterial communities (Zarraonaindia et al., 2015). However, as with variation between

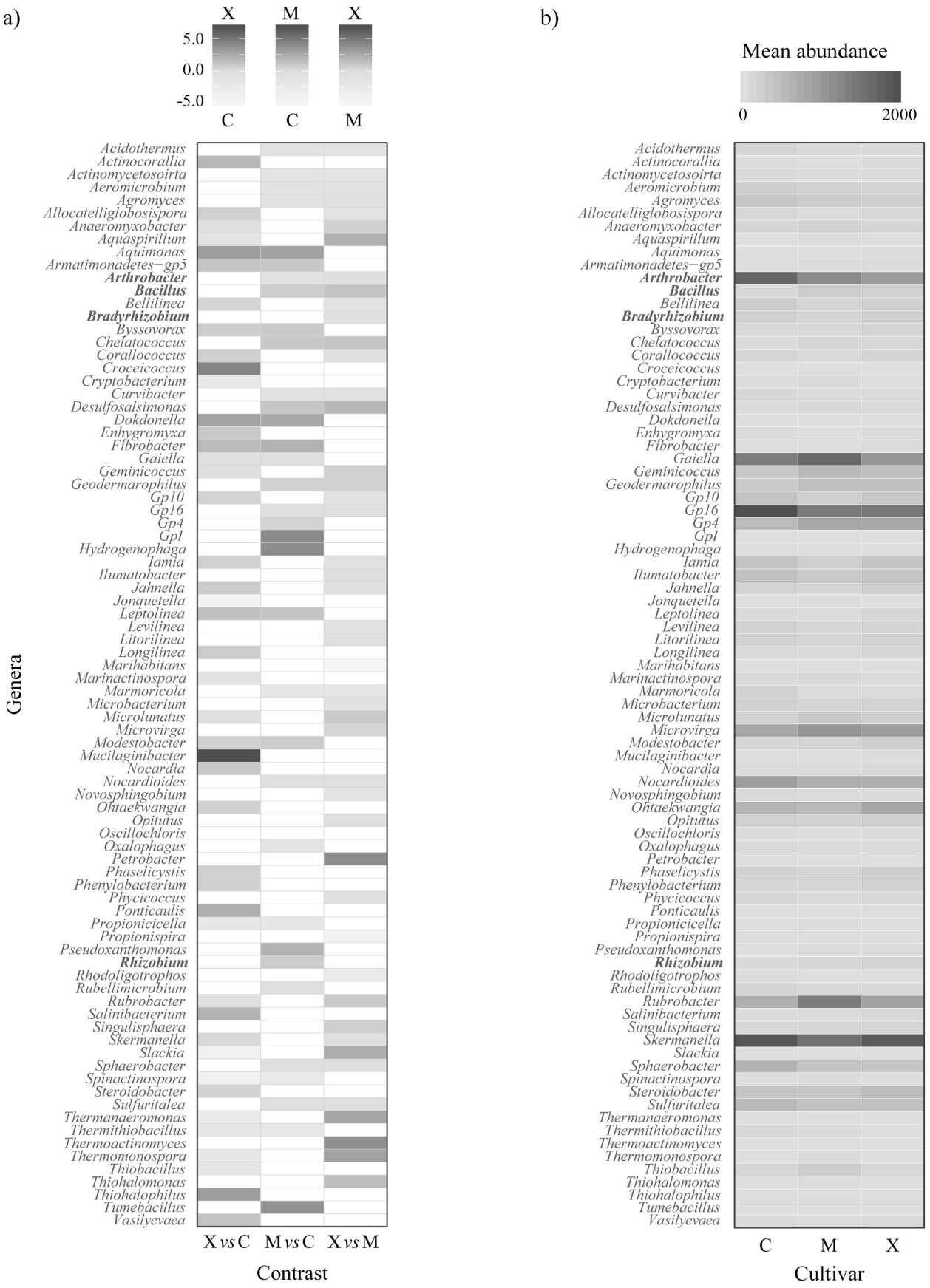

Fig. 5. Variation of individual genera per cultivar. Log2-fold change is shown for each pair wise comparison between different cultivars (a) and mean abundance of each corresponding genus for each cultivar (b). Genera that have members with known biocontrol and/or plant growth promoting properties are in bold. Culivars are as follows: C = Chardonnay, M = Maratheftiko and X = Xynisteri.

cultivars, the effect of plant phenotypic or temporal variability and grapevine associated soil bacterial communities has remained understudied.

5. Conclusion

To our knowledge this is the first study to examine *Vitis vinifera* bacterial communities using this combination of factors, in particular including different cultivars and plant developmental stages. Our results indicate that bacterial communities in grapevines are shaped by both plant-associated effects (i.e. cultivar and plant phenological stage) and environmental variables (i.e. soil management and soil physicochemical properties), and importantly, that these factors have an interactive effect. We recommend that future research on soil bacterial communities in grapevines take this variability into account, as well as its functional implications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2020.103807.

References

Anderson, M.J., Ellingsen, K.E., McArdle, B.H., 2006. Multivariate dispersion as a measure of beta diversity. Ecol. Lett. 9 (6), 683–693. https://doi.org/10.1111/j.1461-0248.2006.00926.x.

Battaglia, 2020. btools: A Suite of R Function for All Types of Microbial Diversity Analyses. R package version 0.0.1. https://github.com/twbattaglia/btools.

Belmonte, S.A., Celi, L., Stahel, R.J., Bonifacio, E., Novello, V., Zanini, E., Steenwerth, K. L., 2018. Effect of long-term soil management on the mutual interaction among soil organic matter, microbial activity and aggregate stability in a vineyard. Pedosphere 28 (2), 288–298. https://doi.org/10.1016/S1002-0160(18)60015-3.

Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68 (1), 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x.

Berlanas, C., Berbegal, M., Elena, G., Laidani, M., Cibriain, J.F., Sagües, A., Gramaje, D., 2019. The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards. Front. Microbiol. 10, 1142. https://doi.org/10.3389/fmicb.2019.01142.

Bokulich, N.A., Thorngate, J.H., Richardson, P.M., Mills, D.A., 2014. PNAS plus: from the cover: microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. Proc. Natl. Acad. Sci. 111, E139–E148. https://doi.org/10.1073/pnas.1317377110.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E., Da Silva, R., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G., Lee, J., Ley, R., Liu, Y., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson II, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C. H., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J. G., 2018. QIIME 2: reproducible, interactive, scalable, and extensible microbiome data science. PeerJ Prepr. 6, e27295v2 https://doi.org/10.7287/peerj.preprints.27295v2.

Burns, K.N., Kluepfel, D.A., Strauss, S.L., Bokulich, N.A., Cantu, D., Steenwerth, K.L., 2015. Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: differentiation by geographic features. Soil Biol. Biochem. 91, 232–247. https://doi.org/10.1016/j.soilbio.2015.09.002.

Burns, K.N., Bokulich, N.A., Cantu, D., Greenhut, R.F., Kluepfel, D.A., O'Geen, A.T., Strauss, S.L., Steenwerth, K.L., 2016. Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: differentiation by vineyard management. Soil Biol. Biochem. 103, 337–348. https://doi.org/10.1016/j.soilbio.2016.09.007.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869.

Campisano, A., Pancher, M., Puopolo, G., Puddu, A., Lòpez-Fernàndez, S., Biagini, B., Yousaf, S., Pertot, I., 2015. Diversity in endophyte populations reveals functional and taxonomic diversity between wild and domesticated grapevines. Am. J. Enol. Vitic. 66, 12–21. https://doi.org/10.5344/ajev.2014.14046.

Capó-Bauçà, S., Marqués, A., Llopis-Vidal, N., Bota, J., Barza, E., 2019. Long-term establishment of natural green cover provides agroecosystem services by improving soil quality in a Mediterranean vineyard. Ecol. Eng. 127, 285–291. https://doi.org/10.1016/j.ecoleng.2018.12.008.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108, 4516–4522. https://doi.org/10.1073/pnas.1000080107.

Carter, M.R., Gregorich, E.G., 2008. Soil Sampling and Methods of Analysis, second ed. CRC Press, Boca Raton.

Chaparro, J.M., Badri, D.V., Vivanco, J.M., 2014. Rhizosphere microbiome assemblage is affected by plant development. ISME J. 8, 790–803. https://doi.org/10.1038/ismej.2013.196.

Chrysargyris, A., Xylia, P., Litskas, V., Mandoulaki, A., Antoniou, D., Boyias, T., Stavrinides, M., Tzortzakis, N., 2018. Drought stress and soil management practices in grapevines in Cyprus under the threat of climate change. J. Water Clim. Change 9 (4), 703–714. https://doi.org/10.2166/wcc.2018.135.

Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D. J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018. A global atlas of the dominant bacteria found in soil. Science 359 (6373), 320–325. https://doi.org/10.1126/science.aap9516.

FAO, 2014. World Reference Base for Soil Resources 2014: International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. FAO, Rome.

Fraga, H., Malheiro, A.C., Moutinho-Pereira, J., Santos, J.A., 2013. An overview of climate change impacts on European viticulture. Food Energy Secur. 1, 94–110. https://doi.org/10.1002/fes3.14.

Friesen, M.L., Porter, S.S., Stark, S.C., von Wettberg, E.J., Sachs, J.L., Marinez-Romero, E., 2011. Microbially mediated plant functional traits. Annu. Rev. Ecol. Evol. Syst. 42, 23–46. https://doi.org/10.1146/annurev-ecolsys-102710-145039.

Gilbert, J.A., van der Lelie, D., Zarraonaindia, I., 2014. Microbial terroir for wine grapes. Proc. Natl. Acad. Sci. U. S. A. 111, 5–6. https://doi.org/10.1073/pnas.1320471110.

Goh, C., Veliz Vallejos, D.F., Nicotra, A.B., Mathesius, U., 2013. The impact of beneficial plant-associated microbes on plant phenotypic plasticity. J. Chem. Ecol. 39, 826–839. https://doi.org/10.1007/s10886-013-0326-8.

Goulet, E., Dousset, S., Chaussod, R., Bartoli, F., Doledec, A.F., Andreux, F., 2004. Water-stable aggregates and organic matter pools in a calcareous vineyard soil under four soil-surface management systems. Soil Use Manag. 20, 318-324. doi: 0.1079/SUM2004263.

Guzmán, G., Cabezas, J.M., Sánchez-Cuesta, R., Lora, Á., Bauer, T., Strauss, P., Winter, S., Zaller, J.G., Gómez, J.A., 2019. A field evaluation of the impact of temporary cover crops on soil properties and vegetation communities in southern Spain vineyards. Agric. Ecosyst. Environ. 272, 135–145. https://doi.org/10.1016/j.agee.2018.11.010.

Hartman, K., van der Heijden, M.G.A., Wittwer, R.A., Banerjee, S., Walser, J.-C., Schlaeppi, K., 2018. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6, 14. https://doi.org/10.1186/s40168-017-0389-9.

Hartmann, A., Schmid, M., van Tuinen, D., Berg, G., 2009. Plant-driven selection of microbes. Plant Soil 321, 235–257. https://doi.org/10.1007/s11104-008-9814-y.

Hatmi, S., Gruau, C., Trotel-Aziz, P., Villaume, S., Rabenoelina, F., Baillieul, F., Aullaffroy, P., Clement, C., Ferchichi, A., Aziz, A., 2015. Drought stress tolerance in grapevine involves activation of polyamine oxidation contributing to improved immune response and low susceptibility to *Botrytis cinerea*. J. Exp. Bot. 66 (3), 775–787. https://doi.org/10.1093/jxb/eru436.

Inceoğlu, Ö., Al-Soud, W.A., Salles, J.F., Semenov, A.V., van Elsas, J.D., 2011. Comparative analysis of bacterial communities in a potato field as determined by pyrosequencing. PLoS One 6 (8), e23321. https://doi.org/10.1371/journal.pone.0023321.

Jiang, Y., Li, S., Li, R., Zhang, J., Liu, Y., Lv, L., Zhu, H., Wu, W., Li, W., 2017. Plant cultivars imprint the rhizosphere bacterial community composition and association

networks. Soil Biol. Biochem. 109, 145–155. https://doi.org/10.1016/j.soilbio.2017.02.010.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30 (4), 772–780. https://doi.org/10.1093/molbev/mst010.
Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: R tools for integrating phylogenies and ecology. Bioinf. 26, 1463–1464. https://doi.org/10.1093/bioinformatics/btq166.
Likar, M., Stres, B., Rusjan, D., Potisek, M., Regvar, M., 2017. Ecological and conventional viticulture gives rise to distinct fungal and bacterial microbial communities in vineyard soils. Appl. Soil Ecol. 113, 86–95. https://doi.org/10.1016/j.apsoil.2017.02.007.
Liu, F., Hewezi, T., Lebeis, S.L., Pantalone, V., Grewal, P.S., Staton, M.E., 2019. Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. BMC Microbiol. 19, 201. https://doi.org/10.1186/s12866-019-1572-x.
López-Piñeiro, A., Muñoz, A., Zamora, E., Ramírez, M., 2013. Influence of the management regime and phenological state of the vines on the physicochemical properties and the seasonal fluctuations of the microorganisms in a vineyard soil under semi-arid conditions. Soil Tillage Res. 126, 119–126. https://doi.org/10.1016/j.still.2012.09.007.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550. https://doi.org/10.1186/s13059-014-0550-8.
Marasco, R., Rolli, E., Fusi, M., Michoud, G., Daffonchio, D., 2018. Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. Microbiome 6, 3. https://doi.org/10.1186/s40168-017-0391-2.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. J., 17(1), 10-12. Doi:10.14806/ej.17.1.200.
McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8 (4), e61217. https://doi.org/10.1371/journal.pone.0061217.
Mendes, L.W., Raaijmakers, J.M., de Hollander, M., Mendes, R., Tsai, S.M., 2017. Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. ISME J. 12 (1), 212–224. https://doi.org/10.1038/ismej.2017.
Mendes, R., Garbeva, P., Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Ecol. 37, 634–663. https://doi.org/10.1111/1574-6976.12028.
Mezzasalma, V., Sandionigi, A., Guzzetti, L., Galimberti, A., Grando, M.S., Tardaguila, J., Labra, M., 2018. Geographical and cultivar features differentiate grape microbiota in Northern Italy and Spain vineyards. Front. Microbiol. 9, 946. https://doi.org/10.3389/fmicb.2018.00946.
Oger, P.M., Mansouri, H., Nesme, X., Dessaux, Y., 2004. Engineering root exudation of *Lotus* toward the production of two novel carbon compounds leads to the selection of distinct microbial populations in the rhizosphere. Microb. Ecol. 47, 96–103. https://doi.org/10.1007/s00248-003-2012-9.
OIV (2019). 2019 Statistical Report on World Vitiviniculture.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.M, Szoecs, E., Wagner, H., 2018. vegan: Community Ecology Package. R package version 2.5-3.
Pingel, M., Reineke, A., Leyer, I., 2019. A 30-years vineyard trial: plant communities, soil microbial communities and litter decomposition respond more to soil treatment than to N fertilization. Agric. Ecosyst. Environ. 272, 114–125. https://doi.org/10.1016/j.agee.2018.11.005.
Price, M.N., Dehal, P.S., Arkin, A.P., 2010. Fasttree 2–approximately maximum-likelihood trees for large alignments. PLoS One 5 (3), e9490. https://doi.org/10.1371/journal.pone.0009490.
R Core Team, 2018. R: A Language and Environment for Statistical Computing. In: R Foundation for Statistical Computing. Austria. URL, Vienna. https://www.R-project.org/.
Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M.L., Gandolfi, C., Casati, E., Previtali, F., Gerbino, R., Cei, F.P., Borin, S., Sorlini, C., Zocchi, G., Daffonchio, D., 2015. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. Env. Microbiol. 17 (2), 316–331. https://doi.org/10.1111/1462-2920.12439.
Ruiz-Colmenero, M., Bienes, R., Eldridge, D.J., Marques, M.J., 2013. Vegetation cover reduces erosion and enhances soil organic carbon in a vineyard in the central Spain. Catena 104, 153–160. https://doi.org/10.1016/j.catena.2012.11.007.
Salinas, M.B., Fardeau, M.-L., Cayol, J.-L., Casalot, L., Patel, M.K.C., Thomas, P., Garcia, J.-L., Ollivier, B., 2004. *Petrobacter succinatimandens* gen. nov., sp. nov., a moderately thermophilic, nitrate-reducing bacterium isolated from an Australian oil well. Int. J. Syst. Evol. Microbiol. 54, 645–649. https://doi.org/10.1099/ijs.0.02732-0.
Santillán, D., Iglesias, A., La Jeunesse, I., Garrote, L., Sotes, V., 2019. Vineyards in transition: a global assessment of the adaptation needs of grape producing regions under climate change. Sci. Total Environ. 657, 839–852. https://doi.org/10.1016/j.scitotenv.2018.12.079.
Six, J., Paustian, K., 2014. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. Soil Biol. Biochem. 68, A4–A9. https://doi.org/10.1016/j.soilbio.2013.06.014.
Stamatakis, A., Chrysargyris, A., 2017. Municipal Solid Waste Compost (MSWC) in pot culture growing media in Greece. In: Tzortzakis, N. (Ed.), Municipal Solid Waste: Management Strategies, Challenges and Future Directions. Nova Science Publishers, New York.
Theodorou, N., Nikolaou, N., Zioziou, E., Kyraleou, M., Kallithraka, S., Kotseridis, Y., Koundouras, S., 2019. Anthocyanin content and composition in four red winegrape cultivars (*Vitis vinifera* L.) under variable irrigation. OENO One 1, 39-51. doi:10.20870/oeno-one.2019.53.1.2366.
Tian, H., Gao, P., Chen, Z., Li, Y., Li, Y., Wang, Y., Zhou, J., Li, G., Ma, T., 2017. Compositions and abundances of sulfate-reducing and sulfur-oxidizing microorganisms in water-flooded petroleum reservoirs with different temperatures in China. Front. Microbiol. 8, 143. https://doi.org/10.3389/fmicb.2017.00143.
Triplett, G.B., Dick, W.A., 2008. No-tillage crop production: a revolution in agriculture! Agron. J. 100 (Suppl3), S153–S165. https://doi.org/10.2134/agronj2007.0005c.
Tzortzakis, N., Chrysargyris, A., Aziz, A., 2020. Adaptive response of a native Mediterranean grapevine cultivar upon short-term exposure to drought and heat stress in the context of climate change. Agronomy 10, 249. https://doi.org/10.3390/agronomy10020249.
Vacheron, J., Desbrosses, G., Bouffaud, M.-L., Touraine, B., Moënne-Loccoz, Y., Muller, D., Legendre, L., Wisniewski-Dyé, F., Prigent-Combaret, C., 2013. Plant growth-promoting rhizobacteria and root system functioning. Front. Plant Sci. 4, 356. https://doi.org/10.3389/fpls.2013.00356.
Vega-Avila, A.D., Gumiere, T., Andrade, P.A., Lima-Perim, J.E., Durrer, A., Baigori, M., Vazquez, F., Andreote, F.D., 2015. Bacterial communities in the rhizosphere of *Vitis vinifera* L. cultivated under distinct agricultural practices in Argentina. Antonie Van Leeuwenhoek 107 (2), 575–588. https://doi.org/10.1007/s10482-014-0353-7.
Vukicevish, E., Lowery, D.T., Úrbez-Torres, J.R., Bowen, P., Hart, M., 2017. Groundcover management changes grapevine root fungal communities and plant-soil feedback. Plant Soil 424 (1–2), 419–433. https://doi.org/10.1007/s11104-017-3532-2.
Wagner, M.R., Lundberg, D.S., del Rio, T.G., Tringe, S.G., Dangl, J.L., Mitchell-Olds, T., 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat. Commun. 7, 12151. https://doi.org/10.1038/ncomms12151.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73 (16), 5261–5267. https://doi.org/10.1128/AEM.00062-07.
Wei, Z., Yang, T., Friman, V.-P., Xu, Y., Shen, Q., Jousset, A., 2015. Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. Nat. Commun. 6, 8413. https://doi.org/10.1038/ncomms9413.
Zapata, C., Deléens, E., Chaillou, S., Magné, C., 2004. Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). J. Plant Physiol. 161, 1031–1040. https://doi.org/10.1016/j.jplph.2003.11.009.
Zarraonaindia, I., Owens, S.M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, A., Mills, D.A., Martin, G., Taghavi, S., van der Lelie, D., Gilbert, J.A., 2015. The soil microbiome influences grapevine-associated microbiota. mBio 6 (2), e02527-14. https://doi.org/10.1128/mBio.02527-14.