Original Research

Influence of Fertilizers and Soil Conditioners on Soil Bacterial Diversity and the Quality of Wine Grape (Cabernet Sauvignon)

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Abstract

Fertilization influences the grape (*V. vinifera*) quality, soil biochemical profiles and bacterial diversity. Twenty-five experiment plots of grape (*V. vinifera* L. cv. Cabernet sauvignon, 4-year-old) were assigned into five groups and treated with four fertilization schedules (inorganic, organic, combined fertilizers, and soil conditioners) or without fertilization (Blank control). Properties of soil chemistry and grape quality were determined, and bacterial diversity was analyzed. Soil organic matter was increased by organic and combined fertilizers; available N, P and K and total N contents were increased by all fertilization schedules. Inorganic fertilizers increased tannin content; organic fertilizers increased total phenols and decreased tannin; combined fertilizers decreased soluble solids; and soil conditioners only increased tannin and decreased the total soluble solids, phenol compounds, titratable acids and sugar-acidity ratio. 16S rRNA sequencing analysis showed *Micrococcaceae*, *Cytophagaceae* and *Streptomycetaceae* abundance was increased by inorganic, organic and combined fertilizers, respectively. In comparison with inorganic fertilizers, soil conditioners reduced the abundance of *Hyphomicrobiaceae*, *Micromonosporaceae*, *Rhodospirillaceae* and *Sphingomonadaceae*. Canonical correspondence analysis showed that soil available N and P as well as grape anthocyanin contents were correlated with *Halomonas*, *Pseudomonas*, *Rhodoplanes*, *Steroidobacter* and *Streptomyces* abundance. Application of fertilizers increased soil fertility and grape berry quality via changing profiles of soil bacteria, including *Streptomycetaceae*, *Hyphomicrobiaceae Micrococcaceae* and *Cytophagaceae* families.

Keywords: bacterial taxonomy; organic fertilizers; vitis vinifera; soil conditioner

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Introduction

Grape (*Vitis vinifera*, *V. vinifera*) is a clonally propagated and worldwide cultivated fruit crop. Wine business holds an important position in national economy. The quality, texture and aroma of wine is prominently influenced by the quality of wine grape [1, 2]. In addition to berry size, picking period, postharvest technology and vinification process, contents of total soluble solids, phenol compounds, tannins, titratable acids and sugar-acidity ratio, which is depended on the titratable acids (tartaric acid and malic acid), also affect the quality of wine grape [1-4]. Wine grape quality is mainly determined by excessive accumulation of sugar and polyphenols that impress the flavor and aroma of wine [4]. What's more, phenols (including tannin, polyphenols and flavonoids) have positive effect on human health, including prevention of cardiovascular disease, anti-hyperglycemic and antioxidant effect [5-7]. Agricultural practices hence purport to improve berry quality and yield [3].

The quality biochemical compounds in grape berry are variable and commonly influenced by various factors, including weather elements, varieties, diseases, fertilizers and postharvest technologies [3, 4, 8]. There is much evidence shows that phenol compounds are easily influenced by varieties, cultural practices, climates and geographical environments [3, 9]. For instance, the proper application of foliar fertilizers significantly increases the content of total phenolic compounds and anthocyanin [3, 10]. Evidence suggests that agricultural practices such as fertilization take important roles in controlling the biosynthesis and total concentrations of valuable traits in grapes.

Applications of inorganic, organic and microbial fertilizer as well as soil conditioners definitely increase the yield and quality of crops [3, 11, 12]. However, the quality of crop is threatening by increased soil problems induced by long-term application of mineral/inorganic fertilizers, including nitrogen (N)-phosphorus (P)-potassium (P) fertilizers [13-15]. Fertilization with organic fertilizers and microbial fermentation-derived soil conditioners is widely used as soil amendments in consideration of long-range benefits to crops and land fertility. Li et al performed a 24-year field experiment and found that the combined organic-inorganic fertilizers increased the contents of soil organic matter and total N, and altered soil bacterial diversity [16]. Similarly, Hou et al showed a 6-year usage of soil conditioners that derive from food waste dynamic rapid fermentation increased soil organic matter and altered the distribution of bacteria [17].

Nowadays, the influence of microbial fertilizers and microbial fermentation-derived soil conditioners on soil bacterial diversity and community is being widely researched. However, there is less information of the difference between them in changing soil bacterial of wine grape. We hypothesized that different fertilization strategies have great influence on fruit taste and soil microorganism. This study performed a comparative analysis to compare the differences in soil bacterial community under different fertilizers (inorganic, organic, combined inorganic-organic fertilizers and soil conditioners). Experiments were performed in a vineyard at the a cultivation base of wine grape in China (Yinchuan, Ningxia). Soil nutrients, wine grape (*V. vinifera* L. cv. Cabernet sauvignon) quality properties, and soil bacterial community structure were investigated and compared. This study would give us new information on making optimal fertilization schedules for improving wine grape quality in China.

Materials and Methods

Field Site

Field experiments were carried out on the wine grape cultivation base of Lilan Chateau, at the eastern foot of Helan Mountain, Yinchuan, Ningxia province, China (longitude 106ºE, latitude 37º~39ºN, altitude 1160 m). This site is under a temperate continental arid

Table 1. The baseline soil chemical parameters.

Parameters	0-20 cm	20-40 cm	40-60 cm
pH	8.32±0.02	8.47±0.05	8.40±0.03
Total salt (g/kg)	0.41±0.01	0.45±0.01	0.46±0.00
Organic matter (g/kg)	10.35±0.96	10.21±1.02	10.14±0.75
Available N (mg/kg)	42.47±5.37	10.97±1.33	2.8±0.02
Available P (mg/kg)	17.73±0.25	4.46±0.03	1.03±0.01
Available K (mg/kg)	163.33±10.25	193.33±13.67	80±10.18
Total P (g/kg)	0.29±0.01	0.24±0.00	0.2±0.01
Total N (g/kg)	0.5±0.01	0.42±0.01	0.17±0.00
Total K (g/kg)	23.3±0.19	22.27±0.27	20.2±0.99

Table 2. The baseline soil physical properties.

Parameters	0-20 cm	20-40 cm	40-60 cm
Water (%)	12.34±1.02	12.07±1.85	12.25±1.03
Capacity (g/cm^3)	1.66±0.05	1.45±0.10	1.37±0.20
Porosity (%)	37.41±2.33	45.42±5.53	48.15±5.82
Saturation capacity (%)	22.37±2.31	29.88±3.33	26.91±2.31
Field capacity (%)	17.72±1.67	21.74±1.95	32.73±3.53
Capillary porosity (%)	29.35±3.57	31.21±2.22	41.53±4.22
Non-capillary porosity (%)	8.05±0.98	14.2±2.53	5.51±0.52

climate with low annual rainfall (~200 mm), high annual evaporation (~1580 mm) and short frost-free period (176 days). The active accumulated temperature during April to September is 3289ºC and annual solar radiation is 6 100 MJ/m^2. The soil here is characterized by laomy sand (sierozem). The soil biochemical and physical parameters are listed in Table 1 and Table 2, respectively.

Experimental Materials and Design

Four-year old plants of *V. vinifera* L. cv. Cabernet sauvignon were used as the experimental materials in our study. All the *V. vinifera* plants were identified by the Germplasm resources center of Agricultural College of Ningxia University. Fertilizations were performed during June 2016 ~ Oct 2017. Grape plants were divided into 25 plots, and each plot counted 60 plants in three lines in north-south direction (n = 20 in each line) with 0.8 m plant spacing and 3.0 m row spacing. Experimental plots were randomly assigned into five groups in a multiple factor randomized block design. Plots in each group were treated with NPK inorganic fertilizer (NPK group), organic fertilizer (Org group), combined mineral-organic fertilizers (Com group) and soil conditioners (derived from natural peat rich of organic matter and humic acid; CS group) or nothing (Blank group), respectively. The fertilizer schedules are shown in Table 3. Fertilizers were mixed with excavated soils and backfilled into holes (60 cm in

depth, 30~35 cm far from plants). All test plots were regularly irrigated with 3000 m^3/hm^2 water per time.

Biochemical Parameters Determination

Root-rhizosphere soil samples were collected in quintuplicate in Aug 2017, about one month before harvest. Soil samples were ground into powder, filtered and dissolved into distilled water (1:3). Soil organic matter (organic carbon) was determined using $K_2Cr_2O_7$ digestion method [18]. Total N and P content was detected using sulfuric acid elimination-Kjeldahl determination method and Vanadium molybdate yellow colorimetry, respectively. Available N, P and K content was determined using alkaline hydrolysis diffusion method, 0.5 mol/L $NaHCO_3$ extraction-colorimetric method and CH_3COONH_4 extraction-flame photometric method, respectively. All methods were performed following the guidelines edited by Bao et al. [19].

Measurement of Wine Grape Berry Quality Properties

Grape berries were harvested in quintuplicate in Sep 2017. Soluble solid content was determined using a MISCO Palm Abbe™ handheld digital refractometer (MISCO PA201, Misco, Solon, OH, USA). Titratable acid, total phenols, and tannin content was detected using NaOH titration method, Folin-Ciocalteu method and Folin-Denis assay, respectively. Anthocyanin

Table 3. The formulas of the five different fertilization schedules.

Composition	Blank	NPK	Org	Com	SC
N (kg/hm^2)	0	343.5	0	181.5	0
P_2O_5 (kg/hm^2)	0	166.5	0	88.5	0
K_2O (kg/hm^2)	0	318	0	165	0
Organic fertilizer (t/hm^2)	0	0	9	4.5	0
Soil conditioner (t/hm^2)	0	0	0	0	3

SC, soil conditioner. Org, organic fertilizers. Com, the combination of inorganic and organic fertilizers. NPK, notes the inorganic nitrogen (N)-phosphorus (P)-potassium (P) fertilizers.

content in grape berry was determined using pH-differential spectrophotometry. All experimental methods were performed according to the recommended methods by Li et al. [20].

DNA Extraction and Preparation Library for 16S rRNA Sequencing

Root-rhizosphere soil samples were collected in triplicate from each plot (overall 75 soil samples). 250 mg soil sample was used for DNA extraction using the MOBIO PowerSoil DNA Isolation Kit (MO BIO Laboratories, CA, USA). DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Amplification was performed using universal primer pairs 515F/806R (with barcode) and a Phusion® High-Fidelity PCR Master Mix kit (New England Biolabs, Ipswich, MA, USA). The 16S rRNA gene V4 and V5 regions were amplified following the conditions: predegeneration at 94ºC for 5 min, followed by 30 cycles of 95ºC for 30 s, 53ºC for 45 s and 72ºC for 50 s, and final extension at 72ºC for 5 min. Equal amount of PCR product from triplicate soil samples of each experiment plot were pooled, and then purified using 2% agarose gel and a PCR purification kit (Qiagen, Chatsworth, CA, USA). A total of 20 pooled DNA samples of five groups (n = 3~5 DNA samples in each group) were got and used for the construction of DNA library following the protocols in a DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, USA). Illumina MiSeq pair-end 300 bp platform was employed for the 16S rDNA sequencing. All the sequence data were uploaded to SRA database with the accession number of SUB5267198.

Data Processing and Analysis

Sequencing data was separated according to the Barcode and PCR primer sequences, which were then depleted. Data splicing and quality filtering were performed using FLASH (v1.2.7; http://ccb.jhu. edu/software/FLASH/), Qiime (v1.9.1; http://qiime. org/scripts/split_libraries_fastq.html) and UCHIME algorithm (http://www.drive5.com/usearch/manual/ uchime_algo.html). Operational taxonomic units (OTUs) clustering was conducted using Uparse software (version 7.0.1001; http://drive5.com/uparse/) with the threshold of 97% identity. The abundance (reads number) of OTUs in each sample was calculated, and OTUs with more than two reads were retained and used for further analysis. The alpha diversity indicators (Chao1, ACE, observed OTUs, Shannon and Simpson) and beta diversity index (Unweighted UniFrac distance) of the sequencing data were calculated. Principal Co-ordinates Analysis (PCoA) of samples was performed based on the Unweighted UniFrac distance. SILVA rRNA database (http://www. arb-silva.de/) that available from the Mothur website (http://www.mothur.org/wiki/RDP_reference_files) was used for the annotation of the OTUs. Taxonomy assignment (phylum ~ species level) was performed using Ribosomal Database Project (RDP) classifier (80% confidence), and the relative abundances of OTUs at different taxonomic levels (phylum~species level) were calculated.

Statistical Analysis

Statistical analysis was performed for the data of biochemical parameters and quality properties. All data were expressed as mean±standard deviation. Differences were analyzed using t test in GraphPad Prism 6. Statistics were done using ANOVA (ANOVA) and t tests. Differences between groups in alpha diversity indicators and OTU relative abundances were analyzed using t test. Canonical correspondence analysis (CCA) was performed to identify the correlation between bacteria diversity and soil biochemical parameters or grape quality properties. Dominant bacteria in each group were identified using LDA Effect Size (LEfSe) analysis. Difference at $p < 0.05$

Table 4. The soil nutrient characteristics of the experiment plots after being treated with different fertilization schedules.

Parameters	Blank	NPK	Org	Com	SC
Organic matter (g/kg)	7.13±0.50	7.02±0.06	8.85±0.22**,&&	8.71±0.46*,&&	7.57±0.30&,^^,§
Available N (mg/kg)	25.58±0.36	27.80±1.08*	32.00±0.37**,&&	27.72±1.18*,^^	26.43±0.50*,^^
Available P (mg/kg)	6.67±0.26	9.76±1.04**	9.48±0.28**	11.47±1.05**,&&,^	7.54±0.36*,&&,^^,§§
Available K (mg/kg)	117.20±0.84	248.30±1.52**	284.80 ±3.03**,&&	263.80±6.06**,&&,^^	176.46±9.94**,&&,^^,§§
Total N (g/kg)	0.47±0.01	0.92±0.01**	1.10±0.01**,&&	1.07±0.01**,&&,^	0.61 ±0.09*,&&,^^,§§
Total P (g/kg)	0.28 ±0.02	0.28±0.01	0.29±0.01	0.28 ±0.02	0.26 ±0.02

Data was expressed as mean±standard deviation from 5 replicated samples. * and **, difference at $p < 0.05$ and $p < 0.01$ vs. Blank group, respectively. & and &&, difference at $p < 0.05$ and $p < 0.01$ vs. NPK group, respectively. ^ and ^^, $p < 0.05$ and $p < 0.01$ vs. Org group, respectively. § and §§, $p < 0.05$ and $p < 0.01$ vs. Com group, respectively. All differences were called by t-test. Blank, control group without fertilization. NPK, treated with NPK inorganic fertilizers. Org, treated with organic fertilizers. Com, treated with combined NPK and organic fertilizers. SC, treated with soil conditioners.

Table 5. The quality indicators of grape berry after being treated with different fertilization schedules.

Parameters	Blank	NPK	Org	Com	SC
Tannin (mg/g)	16.29±1.22	18.74±0.58**	14.87 ±0.29*,&&	16.68 ±0.31&&,^^	18.75 ±0.25*,^^,§§
Anthocyanin (mg/g)	9.70±0.07	9.06±0.04**	10.97±1.35&	8.69±1.07^	7.74±0.09**,&&,^^
Total phenols (mg/g)	1.48±0.71	2.86±0.59	4.02±0.04**,&	2.26 ±0.15&,^^	2.35±0.17^^
Soluble solid (%)	26.58±0.91	25.68 ±0.57	26.30 ±0.59	24.48 ±0.46*,&,^	24.42±0.15**,&,^^
Titratable acid (%)	0.66±0.07	0.69±0.11	0.59±0.05	0.58 ±0.01	0.54±0.01*,&,^,§§

Data was expressed as mean±standard deviation from 5 replicated samples. * and **, difference at $p<0.05$ and $p<0.01$ vs. Blank group, respectively. & and &&, difference at $p<0.05$ and $p<0.01$ vs. NPK group, respectively. ^ and ^^, $p<0.05$ and $p<0.01$ vs. Org group, respectively. § and §§, $p<0.05$ and $p<0.01$ vs. Com group, respectively. All differences were called by t-test. Blank, control group without fertilization. NPK, treated with NPK inorganic fertilizers. Org, treated with organic fertilizers. Com, treated with combined NPK and organic fertilizers. SC, treated with soil conditioners.

and $p<0.01$ was considered as significant and very significant, respectively.

Results

Soil Biochemical Properties

In comparison with blank control, organic and combined fertilizers increased soil organic matter ($p<0.05$, Table 4); all fertilization schedules increased the content of available N, P and K and total N in soil ($p<0.05$). The content of total P was not influenced by fertilization schedules (Table 4). Soil conditioners showed a wild but significant impact on soil biochemical parameters compared with other fertilization schedules.

Wine Grape Berry Quality Properties

Comparing with blank control, soil conditioners and NPK fertilizers ranked the first in increasing grape tannin content ($p<0.05$); organic fertilizer decreased tannin content ($p<0.01$), but ranked the first in increasing grape anthocyanin ($p>0.05$) and total phenols ($p<0.01$). Soil conditioners significantly decreased anthocyanin ($p<0.01$), soluble solid ($p<0.01$) and titratable acid ($p<0.05$) but increased tannin content in grape compared with Blank control (Fig. 4). NPK fertilizers decreased anthocyanin content versus blank control ($p<0.01$; Table 5).

Data of 16S rRNA Sequencing

Illumina sequencing analysis of bacterial 16S rRNA gene V4 and V5 regions produced a total of 28,768 OTUs, including 15,655 OTUs (54.41%) represented by more than two reads (Supplementary Table S1 and Fig. S1). PCA showed the similarity of OTUs sequences in most samples (Fig. 1a), and clustering analysis based on the Euclidean distance indicated there were short distances between samples (Fig. 1b). Only two samples (one in SC group and one in Blank group) were distinct from others. No significant differences were seen in the five alpha diversity indicators among groups due to the overlapped standard deviations (Fig. 2).

The fact that rarefaction curves did not reach to a plateau suggested that further sequencing might generated more data with higher bacterial community richness (Supplementary Fig. S2). Bacterial beta-diversity was shown in Fig. 3. There was no distant difference across samples in the five groups.

Relative Abundance of Dominant Soil Bacterial Taxa at Phylum and Family Level

At the phylum level, *Proteobacteria* (34.07%~44.35%), *Actinobacteria* (24.26%~31.49%) and *Chloroflexi* (7.72%~9.53%) were dominant phyla in all groups (Fig. 4a) and Supplementary Table S2). Pairwise comparison analyses showed NPK fertilizers increase the abundance of *Verrucomicrobia* to a higher level (1.92±0.37%) than Blank (1.07±0.49%, $p<0.05$, Fig. 4b); combined fertilizers reduced *Actinobacteria* abundance to a lower level than NPK fertilizers (5.12±1.51% vs. 8.14±1.27%, $p<0.05$, Fig. 4b).

At the family level, 14 family taxa had the relative abundance of higher than 1% and accounted for a total relative abundance of 30.85%~39.65%. *Gaiellaceae* (2.45%~4.54%), *Hyphomicrobiaceae* (2.49%~5.05%) and *Halomonadaceae* (1.26%~3.65%) were dominant taxa in all groups (Fig. 4c and ˢupplementary Table S2). Compared with blank control, NPK fertilizers induced higher abundance of *Micrococcaceae* (2.45±0.54% vs. 1.41±0.29%, $p<0.05$, Fig. 4d), organic increased *Cytophagaceae* abundance (2.64±0.41% vs. 1.55±0.25%, $p<0.05$) and combined fertilizers increased *Streptomycetaceae* level (2.82±0.29% vs. 1.75±0.14%, $p<0.05$). In comparison with NPK fertilizers, soil conditioners decreased the level of *Hyphomicrobiaceae* (2.49±0.49% vs. 3.64±0.43%, $p<0.05$), *Micromonosporaceae* (0.98±0.21% vs. 1.59±0.41%, $p<0.05$), *Rhodospirillaceae* (1.82±0.74% vs. 2.49±0.46%, $p<0.05$) and *Sphingomonadaceae* (1.28±0.27% vs. 1.70±0.17%, $p<0.01$; Fig. 4d).
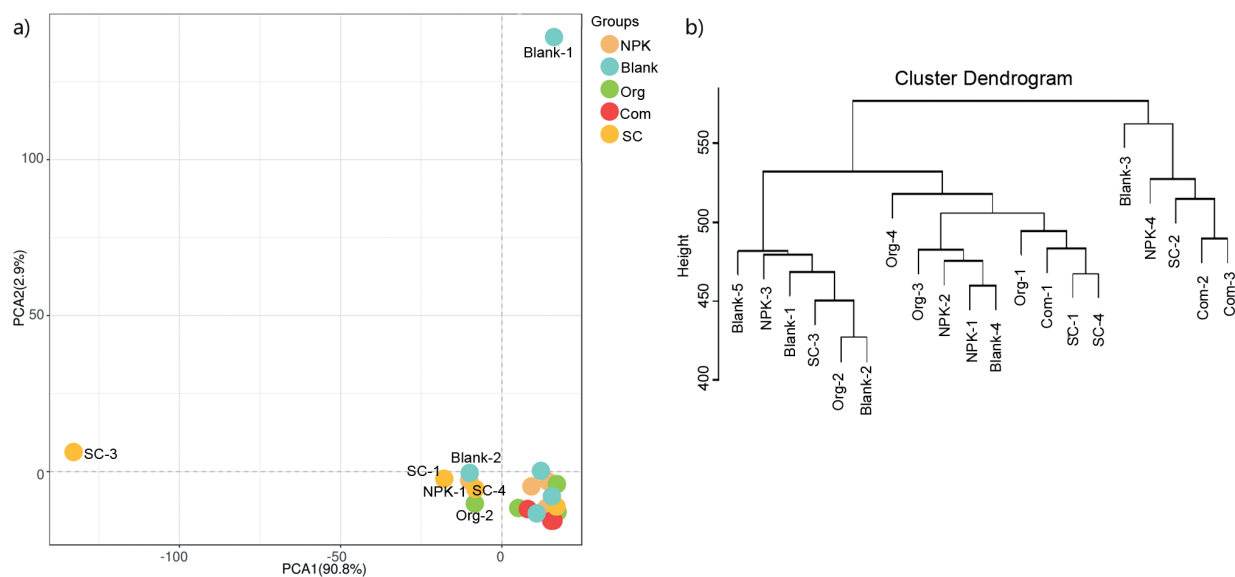
Fig. 1. PCA analysis a) and sample clustering b) of samples used for 16S rRNA sequencing. All analyses were performed based on the relative abundance of OTUs in samples. A, PCA analysis was based on the similarity of OTUs. B, sample clustering was based on the Euclidean distance between OTUs' relative abundances in samples.
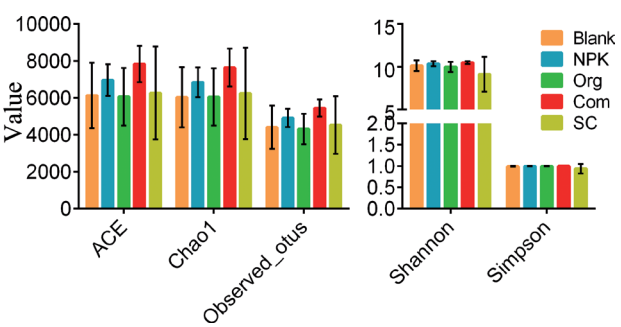

Fig. 2. Alpha diversity indicators of OTUs in five groups.

Dominant Soil Bacterial Taxa at Genus Level

The five genera that had abundance of larger than 1% were *Halomonas* (1.25%~17.41%), *Pseudomonas* (0.34%~2.77%), *Rhodoplanes* (0.98%~2.05%), *Steroidobacter* (1.19%~2.07%) and *Streptomyces* (1.69%~2.75%; Fig. 5a) and Supplementary Table S2). *Rhodoplanes* abundance was significantly increased by organic fertilization (2.05±0.26%, p<0.05) and decreased by soil conditioners (0.97±0.28%, p<0.05) compared with blank control (1.66±0.59%; Fig. 5b),
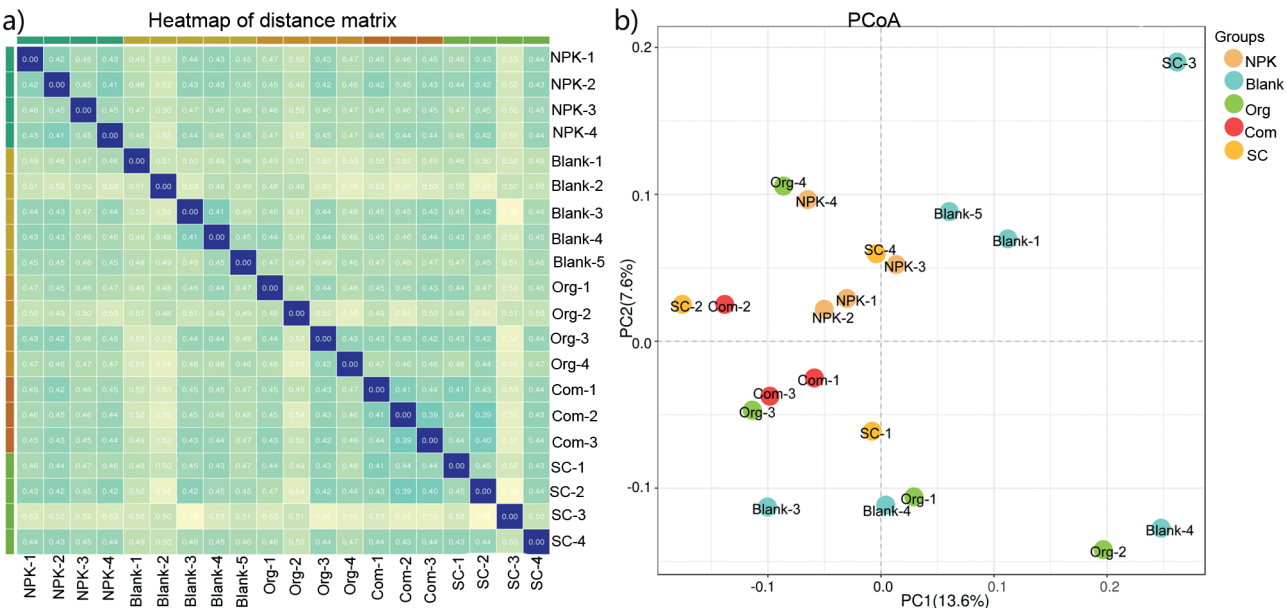
Fig. 3. Beta diversity analysis of OTUs in all samples sequenced. a) heatmap of samples distance matrix of OTUs. b) PCoA of OTUs. Both analyses were based on the Unweighted UniFrac distance.

a)

b)

c)

d)
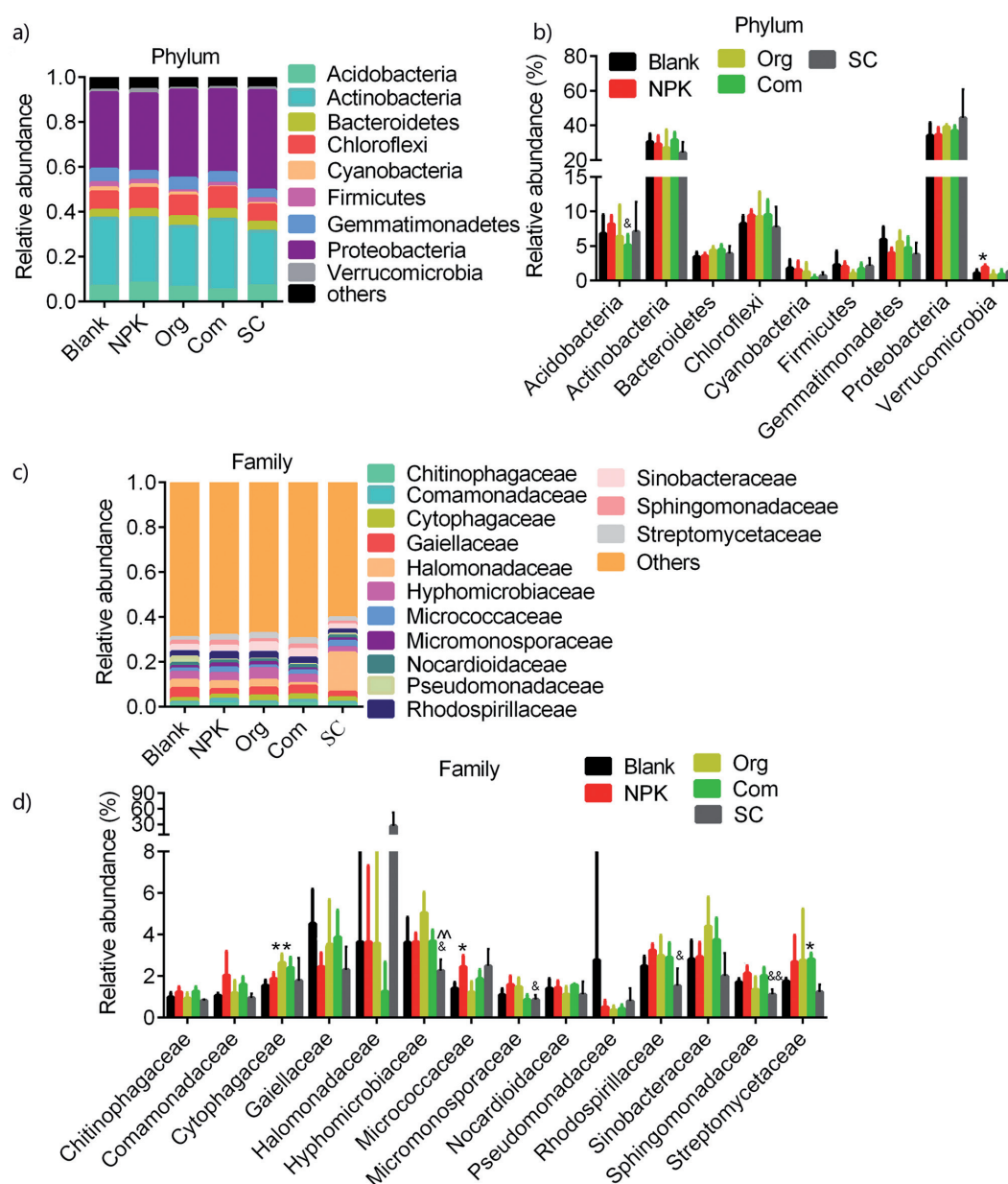
Fig. 4. Relative abundance of the dominant bacteria at the phylum and family level. a) and b), the stacks and statistical analysis for OTUs' relative abundance of the dominant phyla, respectively. c) and d), the stacks and statistical analysis for OTUs' relative abundance of the dominant family, respectively. Data was expressed as mean±standard deviation from 5 replicated samples. * and **, $p<0.05$ and $p<0.01$ vs. Blank group, respectively. & and &&, $p<0.05$ and $p<0.01$ vs. NPK group, respectively. ^^ $p<0.01$ vs. Org group, respectively. All differences were called by t-test.

respectively. *Steroidobacter* abundance was obviously increased by combined fertilizers compared with blank control (2.76±0.29% vs. 1.69±0.16%, $p<0.05$; Fig. 5b).

CCA and Determinants of Soil Biochemical Parameters

Fig. 6 shows the CCA for the 5 dominant genera and soil biochemical parameters. We found that the community diversities of these genera were correlated with the contents of soil available P and N (Fig. 6a). In addition, the diversities were related with tannin and anthocyanin contents in wine grape (Fig. 6b).

Discussion

Among the valuable factors influencing wine flavor and aroma, total phenol compounds extracted from the seed and skin of grapes as well as sugar in berry are the major elements responsible for wine quality [4]. The accumulation of total phenols in grape skin and sugar/soluble solid indicates the improvement of grape quality. We found the 2-year fertilization schedules of organic and combined fertilizers significantly improved soil biochemical properties including the contents of organic matter, available N, P and K and total N (Table 4). In addition, the increased amount of tannin
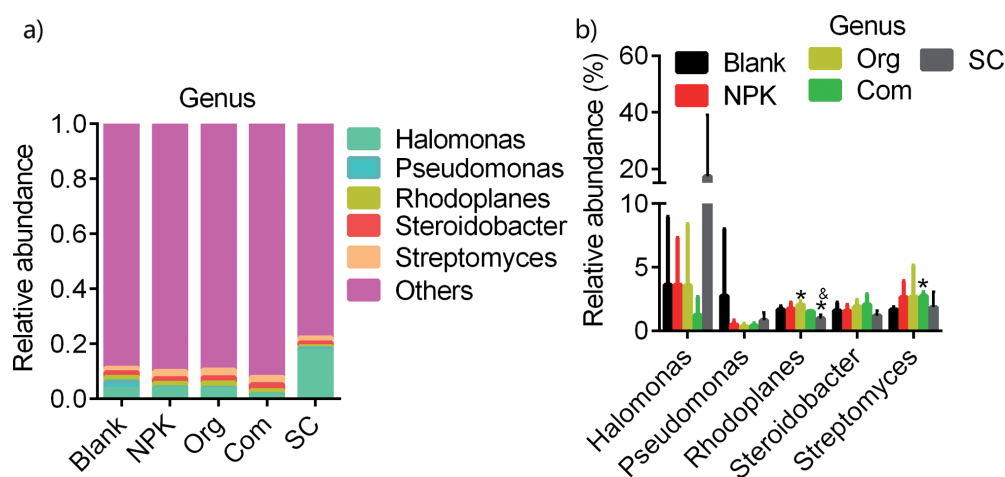
Fig. 5. The stacks a) and statistical analysis b) for OTUs' relative abundance of the dominant bacterial genera. * p<0.05 vs. Blank group, and & p<0.05 vs. NPK group by t-test.

and total phenols in wine grape berry juice confirmed the promotion of grape quality by fertilization schedules.

Grape tannin may contain the precursors of varietal thiols and increased the contents of free thiols that contribute to wine aroma. Larcher et al revealed that the higher contents of tannin in grape berry increased the level of free thiols in wine [21, 22]. The increased contents of tannin in grape berry treated by NPK fertilizers and soil conditioners might suggest the improved aroma of wine by these two schedules. Our study here showed that organic fertilizers only increased total phenols but decreased tannin, NPK fertilizers increased tannin content but decreased anthocyanin content. Four fertilization schedules showed different

influences on grape quality. The related molecular mechanisms underlying quality improvement in grape might be different.

16S rRNA sequencing analysis demonstrated the different response of soil bacterial community to fertilization schedules. We identified that several functional soil bacteria, including phyla *Proteobacteria*, *Actinobacteria* and *Chloroflexi*, as well as families *Gaiellaceae*, *Hyphomicrobiaceae* and *Halomonadaceae* were dominant taxa in soil samples under different treatment. The abundances of them were influenced by fertilizations. For instance, NPK fertilizers increased *Micrococcaceae* abundance, organic and combined fertilizers increased the abundance of *Cytophagaceae* and *Streptomycetaceae*, respectively, compared with
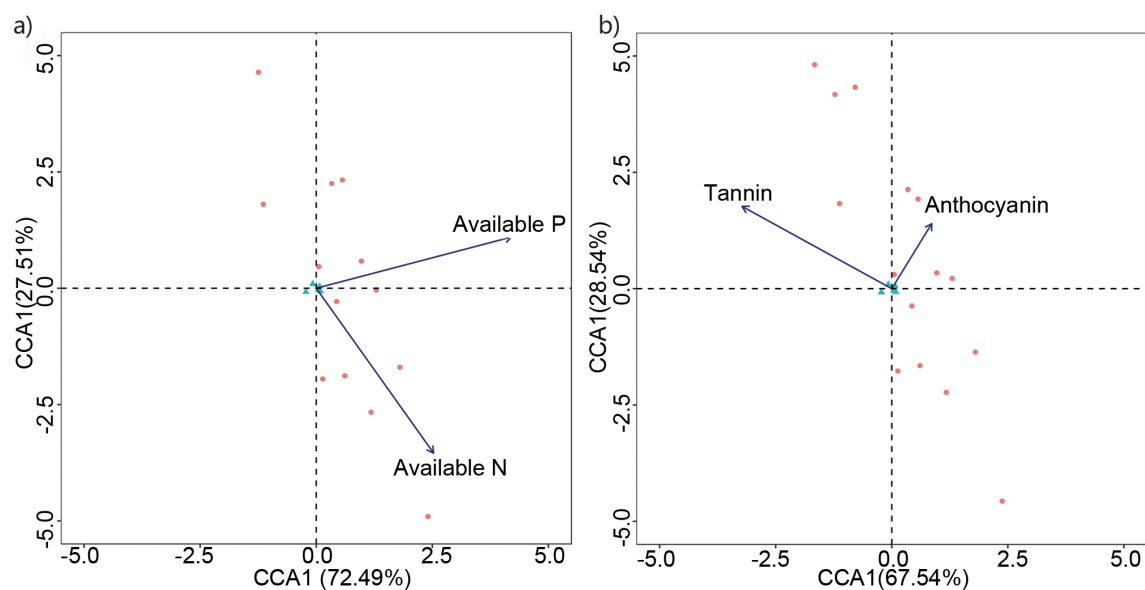

Fig. 6. Canonical correspondence analysis (CCA) analysis of dominant genera. CCA analysis was performed based on unweighted UniFrac distances of genus taxa abundance in samples. a) CCA analysis quantifying the soil biochemical parameters. b) CCA analysis quantifying the wine grape berry quality properties. Line length indicates the strength relative to other variables.

control. *Micrococcaceae* is a sodium polyacrylate and plant growth-promoting bacterium that promotes plant growth and yield [23]. *Cytophagaceae* family is the largest *Bacteroidetes* phylum that consists of a large number of genera (>31) and species (>80) [24-26]. *Cytophagaceae* family member is characterized by predominant respiratory quinone MK-7, major polar lipid phosphatidylethanolamine, 40 mol%~50 mol% DNA G+C content and iso-$C_{15:0}$, $C_{16:1\omega5c}$, and summed feature 3 ($C_{16:1\omega7c}$ and/or $C_{16:1\omega6c}$) cellular fatty acids [25, 27-29]. Various functions of *Cytophagaceae* bacteria have been reported till now, including nitrogen-fixing [28], proteins and polysaccharides digestion [24]. *Streptomycetaceae* family members have important functions in degradation of recalcitrant substances, including xylan, lignin, cellulose and lignocellulose [30-32]. *Streptomycetaceae* members are widely distributed in soils and are closely correlated with decomposition and usage of soil organic matter [30-32]. The relative abundance of *Micrococcaceae*, *Cytophagaceae* and *Streptomycetaceae* was increased by NPK fertilizers, organic fertilizers and/or combined fertilizers. At the genus level, we found the increased *Rhodoplanes* (*Hyphomicrobiaceae* family) and *Streptomyces* (*Streptomycetaceae* family) abundance by organic and combined fertilizers, respectively. The abundant levels might be associated with the increased organic substances in soils, and NPK and organic fertilizers might have improved the active function of these bacteria in soils.

It was interesting that soil conditioners decreased the abundance of *Hyphomicrobiaceae*, *Micromonosporaceae*, *Rhodospirillaceae* and *Sphingomonadaceae* families and *Rhodoplanes* genus (*Hyphomicrobiaceae* family) compared with NPK fertilizers. This was consistent with the lower soil biochemical parameters in SC group than those in Com, Org and NPK groups (Fig. 4 and Table 4), and also linked to the lower contents of anthocyanin, total phenols and soluble solid in wine grape berry (Table 5). Using CCA quantifying, we found the total abundance of the 5 genera including *Halomonas* (*Halomonadaceae* family), *Pseudomonas* (*Pseudomonadaceae* family), *Rhodoplanes* (*Hyphomicrobiaceae* family), *Steroidobacter* (*Sinobacteraceae* family) and *Streptomyces* (*Streptomycetaceae* family) was correlated with the contents of soil available N and P (Fig. 6). In addition, the contents of grape anthocyanin and tannin were associated with the abundance of these 5 genera. These results suggested that fertilization schedules influenced grape quality via changing profiles of soil bacterial taxonomy.

Conclusions

In conclusion, we confirmed the influence of different fertilization schedules on the properties of soil biochemics and wine grape berry quality.

The soil conditioners only increased tannin content, and decreased other quality indicators. Application of fertilizers increased soil fertility and grape berry quality via changing profiles of soil bacteria, including *Streptomycetaceae*, *Hyphomicrobiaceae* *Micrococcaceae* and *Cytophagaceae* families. The genera including *Halomonas*, *Pseudomonas*, *Rhodoplanes*, *Steroidobacter* and *Streptomyces* were correlated with the contents of grape berry anthocyanin and tannin. Fertilization schedules had important effects on the grape quality via changing profiles of soil bacterial diversity.

Conflict of Interest

The authors declare no conflict of interest

Supplementary Material

Supplementary Tables and Figures are available online http://www.pjoes.com/SuppFile/132312/5149/76d f73a829dba4ad478282ee47aed5c3/

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