SOIL MICROBIAL POPULATION STRUCTURES IN GRAPE (VITIS VINIFERA) RHIZOSPHERE IN RESPONSE TO A SHORT-TERM CALCIUM FERTILIZER FIELD EXPERIMENT

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Calcium (Ca) is one of the important elements needed for grape (Vitis vinifera) growth, development and resistance to abiotic stresses. We performed this study to explore the alteration in soil bacterial community in grape in response to Ca-fertilization. Four-year-old grape plants (cv. sauvignon) were fertilized with 0 (control, CK), 64.74 (Ca1), 129.74 (Ca2), 194.22 (Ca3), 258.96 (Ca4) and 323.69 kg/hm² (Ca5) Ca(NO₃)₂·4H₂O during the diluting and turning periods for two months. Soil biochemical parameters (eg. available nitrogen, phosphorus, and potassium, total nitrogen and phosphorus, and organic matter) as well as grape berry quality indicators (eg. soluble solid, tannin, titratable acid, anthocyanins and total phenols) were measured. The changes in soil bacterial community in response to Ca-fertilizers were detected by Illumina 16S rRNA gene (V3-V4 region) sequencing. Soil organic matter, available nitrogen, phosphorus and potassium and total nitrogen and phosphorus were increased by Ca1, Ca2, Ca4 and Ca5 fertilizations compared with control. Ca-fertilizers increased the tannin and anthocyanin contents in grape. We observed that the Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria and Gemmatimonadetes were abundant bacteria in all groups. The high Ca (Ca5) fertilization practice decreased Gemmatimonadetes phylum and increased Nitrospirae phylum and Gemmati monadaceae family, respectively. Ca-fertilizers induced changed in soil fertility and grape quality were related with the altered soil Gemmatimonadaceae, Nitrospirae and Actinobacteria bacteria, which might also be related to the increased defense or tolerance to abiotic stresses in grape.

Keywords: 16S rRNA gene sequencing; Rhizosphere bacteria; Calcium; Vitis vinifera.

INTRODUCTION

Calcium (Ca) is one of the important elements needed for grape (Vitis vinifera). Ca mainly exists in the cell wall in the form of pectin calcium, which can deposit polysaccharides, maintain the firmness and stabilization of cell wall, promote chlorophyll formation and enhance the resistance to pests and diseases (Hepler et al., 1985; Bush, 1995; Liu et al., 1998; Davarpanah et al., 2018). In addition, Ca also acts as a central regulator of plant growth and development via the Ca²⁺ signaling or regulation of plant enzymes (Bush, 1995; Snedden et al., 1995; Liu et al., 1998; Hepler 2005; Kudla et al., 2010). Ca can promote the conversion and absorption of nitrate nitrogen (NO₃⁻) in the soil, converse the insoluble phosphorus (P) and potassium (K) into soluble nutrients (available P and K).

In grape, the Ca-fertilizers have efficacy on the formation of sugar, the fixation of carbon dioxide and the formation of aromatic compounds (Utke et al., 1964; Yamaguchi et al., 1990). Vitis. vinifera is a clonally propagated and worldwide cultivated fruit crop due to the rising position of wine business in national economy. The quality, texture and aroma of wine are determined by the ecological environment and agricultural practices (Yuyuen et al., 2015; Urcan et al., 2016; Kok et al., 2017; Mencarelli et al., 2018). It has been reported that the foliar Ca fertilization reduced fruit cracking including in grape, lychee, and pomegranate (Huang et al., 2003; Yingcui, 2008; Zhu et al., 2017). The quality indicators, like total sugar, polyphenols, soluble solids, phenol compounds, tannins, titratable acids and sugar-acidity ratio, predicate and finally determine the quality of wine grape berry (Yuyuen et al., 2015; Mencarelli et al., 2018). These indicators are variable and easily influenced by factors like weather elements (including temperature and sunshine), varieties, diseases and fertilizers like Ca (Leeuw et al., 2014; Urcan et al., 2016; Kok et al., 2017; Huang et al., 2018). For instance, the content and composition of phenol compounds are diverse in varieties, and are easily influenced by cultural practices, climates and geographical environments (Mattivi et al., 2010; Kok et al., 2017). The soluble solids, vitamin Cc, soluble sugar as well as grape weight and firmness could be increased by foliar Ca fertilization (Huang et al., 2018).
Nowadays, the influence of the applications of inorganic or organic fertilization practices on soil microbial communities rising is the hot issues for the long-term development and management of land resources. The soil biochemical features, crop yield and quality are reported to be correlated with the rhizosphere bacteria (Sessitsch et al., 2001; Chu et al., 2007), which help plants to tolerate abiotic stress (Yang et al., 2009). Rhizosphere bacteria control the crop yield and quality, plant growth and development via modulating the root metabolism, absorption, conversion and tolerance to abiotic stresses (Yang et al., 2009; DeBruyn et al., 2011; Dubey et al., 2019; Ullah et al., 2019). However, there is less information on the Ca-fertilization induced alteration in rhizosphere bacteria of grape. This study was performed to explore the alteration in soil bacterial community in response to Ca-fertilization. The effect of Ca-fertilization on rhizosphere bacteria, soil fertility, and the quality indicators of wine grape (V. vinifera cv. Cabernet sauvignon) was determined. This study would give novel insights into the fertilization practice for managing grape quality in view of the rhizosphere bacteria.

MATERIALS AND METHODS

Experimental field condition: All experiments were carried out at the wine grape planting base of Lilan Chateau, at the eastern foot of Helan Mountain, Minning town, Yongning county, Yinchuan city, Ningxia province, China (longitude 106° E, latitude 37° ~ 39° N, altitude 1160 m). This site is under temperate arid climate characterized by low rainfall (~200 mm annually), high evaporation (~1580 mm annually), high total solar radiation (~6100 MJ/km² annually) and short frost-free period (176 days). The active accumulated temperature during April to September is 3289°C. The soil here is alkaline loamy sand with very low fertility level. The soil biochemical and physical parameters are shown Table 1.

V. vinifera materials and experimental design

Four-year old V. vinifera cv. Cabernet sauvignon plants were used as experimental plant materials. Plants were treated with Ca-fertilizations annually during July 2018 and Oct 2019. Plants were planted in north-south direction (n = 20 in each line) with 0.8 m ×3.0 m planting space. All plants were fertilized with by root-irrigation (3000 m³/hm²). For different fertilization strategies, grape plants were divided into 18 plots randomly (6 groups x 3 replications), each group was fertilized using Ca(NO₃)₂·4H₂O with 0 (control, CK), 64.74 (Ca1), 129.74 (Ca2), 194.22 (Ca3), 258.96 (Ca4) and 323.69 kg/hm² (Ca5) during the fruit expanding period and fruit color period annually.

Soil biochemical parameters measurement

Root rhizosphere soil samples (30-40 cm depth) were dried, grinded, filtered and dissolved using distilled water (1: 3). The biochemical parameters in soil samples were detected using corresponding methods as described by Bao et al. (BaoS.D 2000). K₂Cr₂O₇ digestion methods were used for determination of organic matter (organic carbon). Available N, P and K content was determined using alkaline hydrolysis diffusion methods, 0.5mol/L NaHCO₃ extraction-Mo-Sb colorimetry and CH₃COONH₄ extraction-flame photometric methods, respectively. Total N and P content were detected using H₂SO₄-H₂O₂ digestion-Nessler’s reagent methods and vanadium molybdate yellow colorimetry, respectively. Each experiment was performed with five repeated samples in each group.

Quality properties parameters measurement

Grape berries were harvested during veraison at Sep 2017. All quality properties parameters were detected using the methods described by Li (Li et al., 2000). Soluble solid content was determined using a palm Abbé™ handheld digital refractometer (MISCO PA201, Misco, USA). Titratable acid, tannin content and total phenols was detected using NaOH titration methods, Folin-Denis assay and Folin-Ciocalteu methods, respectively. The pH-differential spectrophotometry was performed to determine the anthocyanin content in grape berry. Five repeated samples were used for each experiment in each group.

Soil bacterial DNA extraction and Illumina sequencing

Three root rhizosphere soil sample sat depth of 30-40 cm were collected from each experimental plot (overall 90 soil samples, 250 mg of each plot). A MOBIO Power Soil DNA Isolation Kit (MO BIO Laboratories, USA) was used to extract the bacterial DNA in soil samples according to the manufacturer’s instructions. DNA quality inspection was performed using Nano Drop ND-1000 spectrophotometer (Nano Drop Technologies, USA). DNA samples were used for PCR amplification using 51F/806R universal primer.

Table 1. The baseline chemical parameters of soil in our test plots at the beginning of experiments.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0-20 cm</th>
<th>20-40 cm</th>
<th>40-60 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.32±0.00  a</td>
<td>8.47±0.01 a</td>
<td>8.40±0.00 b</td>
</tr>
<tr>
<td>Organic matter (g/kg)</td>
<td>6.26±0.22 a</td>
<td>5.78±0.34 b</td>
<td>4.82±0.16 c</td>
</tr>
<tr>
<td>Available N (mg/kg)</td>
<td>24.03±0.18 a</td>
<td>21.27±0.56 b</td>
<td>13.93±0.35 c</td>
</tr>
<tr>
<td>Available P (mg/kg)</td>
<td>13.26±0.72 a</td>
<td>8.07±0.39 b</td>
<td>4.09±0.68 c</td>
</tr>
<tr>
<td>Available K (mg/kg)</td>
<td>223.33±7.42 a</td>
<td>183.84±2.85 b</td>
<td>117.62±4.57 c</td>
</tr>
<tr>
<td>Total N (g/kg)</td>
<td>0.48±0.02 a</td>
<td>0.44±0.01 b</td>
<td>0.28±0.01 c</td>
</tr>
<tr>
<td>Total P (g/kg)</td>
<td>0.28±0.01 a</td>
<td>0.25±0.01 b</td>
<td>0.17±0.01 c</td>
</tr>
</tbody>
</table>

The significant difference between groups are noted by different lowercase letters in the same column (P<0.05).
pains (515F: 5’-GTGCCAGCMGCGCCGTTAA-3’, 806R: 5’-XXXXXGGACTACHVGGGTWTCTA AT-3’) for the V3and V4regions in 16S rRNA gene. Trans Gen AP221-02 Trans Start Fast pfu DNA polymerase (Trans Gen Biotech, China) was used for PCR amplification. Then, equal amounts of the DNA samples within one plot were pooled, followed with purification and DNA library construction (DNA PCR-Free Sample Preparation Kit; Illumina, USA). 16S rRNA gene sequencing was conducted on the Illumina MiSeq platform with a pair-end (PE) 300 bp strategy.

**Data processing and analysis**

Sequencing data in the format of Fast q were processed using Trimmomatic (Bolger et al., 2014) and Pearson (p<0.0001) (Zhang et al., 2013) for removing the reads <50 bp and barcode reads, respectively. Data splicing and quality filtering were performed as usually using FLASH (http://ccb.jhu.edu/software/FLASH/). Chimera reads were removed using usearch program (Alloui et al., 2015), followed by filtering using mothur to remove tags with short length (<200 bp) (Yang et al., 2014). UParse software (version 7.0.1001; http://drive5.com/uparse/) (Edgar, 2013) was used for the clustering of operational taxonomic units (OTUs) with 97% identity. The abundance of OTUs in each sample was calculated, and singleton OTUs were removed. Rarefaction curves were presented using mothur (Yang et al., 2014). The alpha diversity indicators (Chao1, observed OTUs, PD whole curves) were presented using mothur (version v.1.8) (http://qiime.org/scripts/alpha_rarefaction.html). Accordingly, the Principal Component Analysis (PCA) was performed for the samples. For the annotation of the OTUs’ taxonomy, the SILVA database (http://www.arb-silva.de/) was queried. The relative abundances of OTUs at each taxonomic level were calculated and taxonomy assignment was performed using Ribosomal Database Project (RDP) classifier with 80% confidence. Genetic distance UPGMA (Unweighted pair group method with arithmetic mean) algorithm was used for constructing the construct cluster analysis tree (Stefan Van Dongen et al., 1996).

**Statistical analysis:** All data of biochemical parameters and quality properties were expressed as the mean ± standard deviation (SD). GraphPad Prism 6 was used for statistical analysis. Differences among groups were analyzed using the two-way ANOVA. Different taxonomy in groups was identified using Kruskal-Wallis test. Difference at the level of p<0.05 was considered as significant. LDA Effect Size (LEfSe) analysis was performed to identify the dominant bacteria in each group.

**RESULTS**

Ca-fertilizers changed the soil fertility and grape quality:
The biochemistry analysis showed that Ca-fertilizers changed the contents of soil organic matter, available N, P, and K as well as total N and P contents (Table 2) compared with control. The soil organic matter and total N contents in Ca1, Ca2, Ca3, and Ca5 group were decreased compared with control (p<0.05), while Ca4 did not influence the content of soil organic matter and total N. The soil available N was reduced by Ca1, Ca2, Ca3, and Ca4 fertilization practices (p<0.05), but increased by Ca5 fertilization practice (p<0.05). Ca-fertilizers increased soil available N and total P (p<0.05). In addition, Ca4 fertilization practice increased the content of available P in soil.

**Table 2. Effect of Ca fertilization practices on soil chemical properties.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Organicmatt (g/kg)</th>
<th>Available N (mg/kg)</th>
<th>Available P (mg/kg)</th>
<th>Available K (mg/kg)</th>
<th>Total N (g/kg)</th>
<th>Total P (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>7.26 ± 0.22c</td>
<td>25.59 ± 0.13c</td>
<td>6.62 ± 0.11c</td>
<td>217.20 ± 0.58c</td>
<td>0.46 ± 0.00c</td>
<td>0.28 ± 0.01c</td>
</tr>
<tr>
<td>Ca1</td>
<td>7.89 ± 0.01ab</td>
<td>26.69 ± 0.38ab</td>
<td>7.67 ± 0.22b</td>
<td>213.60 ± 0.24c</td>
<td>0.46± 0.00c</td>
<td>0.33 ± 0.00b</td>
</tr>
<tr>
<td>Ca2</td>
<td>8.00 ± 0.08a</td>
<td>25.68 ± 0.35c</td>
<td>7.69± 0.15b</td>
<td>239.00 ± 0.45c</td>
<td>0.55 ± 0.01s</td>
<td>0.27 ± 0.00c</td>
</tr>
<tr>
<td>Ca3</td>
<td>7.63 ± 0.06abc</td>
<td>27.29 ± 0.49a</td>
<td>5.59 ± 0.00d</td>
<td>222.00 ± 0.45d</td>
<td>0.52 ± 0.00a</td>
<td>0.24 ± 0.00d</td>
</tr>
<tr>
<td>Ca4</td>
<td>7.59 ± 0.12bc</td>
<td>28.05± 0.30a</td>
<td>8.24 ± 0.04a</td>
<td>241.00 ± 0.45d</td>
<td>0.45 ± 0.02c</td>
<td>0.43 ± 0.00a</td>
</tr>
<tr>
<td>Ca5</td>
<td>7.65 ± 0.09ab</td>
<td>27.07 ± 0.86ab</td>
<td>6.85± 0.07c</td>
<td>257.00± 0.31a</td>
<td>0.51 ± 0.00b</td>
<td>0.32 ± 0.00b</td>
</tr>
</tbody>
</table>

The significant difference between groups are noted by different lowercase letters in the same column (P<0.05).

**Table 3. Effect of Ca fertilization practices on the quality of wine grape berry.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Tannin (mg/g)</th>
<th>Anthocyanin (mg/g)</th>
<th>Total Phenols (mg/g)</th>
<th>Soluble Solid (%)</th>
<th>Titratable acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>13.81 ± 0.22c</td>
<td>7.50 ± 0.03d</td>
<td>16.02 ± 0.21c</td>
<td>0.56 ± 0.14c</td>
<td>0.62 ± 0.01c</td>
</tr>
<tr>
<td>Ca1</td>
<td>15.75 ± 0.45b</td>
<td>7.39 ± 0.09b</td>
<td>18.37 ± 0.17b</td>
<td>0.44 ± 0.19b</td>
<td>0.74 ± 0.01b</td>
</tr>
<tr>
<td>Ca2</td>
<td>15.43 ± 0.13b</td>
<td>7.43 ± 0.28b</td>
<td>19.37 ± 0.27b</td>
<td>0.48 ± 0.23b</td>
<td>0.73 ± 0.01b</td>
</tr>
<tr>
<td>Ca3</td>
<td>16.57 ± 0.21a</td>
<td>8.37 ± 0.03a</td>
<td>20.48 ± 0.08a</td>
<td>0.56 ± 0.17b</td>
<td>0.76 ± 0.01a</td>
</tr>
<tr>
<td>Ca4</td>
<td>15.85 ± 0.27b</td>
<td>6.46± 0.15c</td>
<td>18.70 ± 0.23bc</td>
<td>0.52 ± 0.15b</td>
<td>0.74 ± 0.00b</td>
</tr>
<tr>
<td>Ca5</td>
<td>13.64 ± 0.20c</td>
<td>7.30 ± 0.07b</td>
<td>16.87 ± 0.43d</td>
<td>0.50 ± 0.21ab</td>
<td>0.71 ± 0.01b</td>
</tr>
</tbody>
</table>

The significant difference between groups are noted by different lowercase letters in the same column (P<0.05).
In grape berry, the contents of tannin, anthocyanin, total phenols and titratable acids were increased by all Ca fertilization practices (Table 3), while fertilizations decreased the content of soluble solid. Ca3 fertilization practice increased the content of tannin, anthocyanin, total phenols and titratable acids significantly (p<0.05), and did not change the contents of soluble solid. 

**Summary of 16S rRNA sequencing:** Illumina 16S rRNA gene sequencing generated a total of 1,211,295 raw tags, corresponding to 1,201,758 clean tags with an average number of 40,058 tags per sample. The data had been uploaded to SRA database and available with the access of PRJNA59788. The distribution of tag length showed that the length of 99.04% tags was ranged from 400 bp to 460 bp (Figure S1a). A total 18,252 non-overlapping tags corresponding to 5,883 unique OTUs were identified, with an average number of 2,500.3 tags per sample, ranging from 2398 to 2621 OTUs per sample (Table S1). The rarefaction curves of samples sequenced showed deeper sequencing might yield more OTUs (Figure S1b), while Shannon-Wiener curves showed the Illumina sequencing data was sufficient for diversity analysis, and deeper sequencing will not enhance the bacterial diversity (Figure S1c). There were no differences in the alpha diversity indicators including Cha01, observed OTUs, PD whole tree and Shannon among groups (Figure 1). There was no difference in the unweighted_unifrac_distance across groups (Figure 2a), and samples had diverse distances (Figure 2b). PCA (Figure 2c) and the unrooted newick-formatted tree of samples sequenced based on the UPGM showed that samples were not discriminant in Ca1-5 groups (Figure 2d).

**Identification of the differential bacteria in groups:** Based on the abundance of OTUs’ reads, we identified that there were 862 differentially expressed non-overlapping OTUs in the six groups, including 684, 682, 712, 682 and 720 OTUs in Ca1, Ca2, Ca3, Ca4 and Ca5 group in comparison with CK, respectively. Different taxonomies were identified in SILVA database. 

- **Proteobacteria** (20.18 ~25.35%), **Actinobacteria** (21.32~24.24%), **Chloroflexi** (14.79~17.17%), **Acidobacteria** (15.21~18.03%), **Gemmatimonadetes** (6.97~9.59%) and **Bacteroidetes** (2.64~4.42%) were the dominant phyla at the phylum level (Figure 3a and supplementary Table S2). The major bacterial composition was not influenced by the Ca fertilization practices in our study (Figure 3).

We confirmed the influence of short-term Ca fertilization practices, especially Ca5, decreased the abundance of Gemmatimonadetes, but increased that of the Nitrospirae (p<0.05, Figure 3b and supplementary Table S2). In addition, we observed that Gemmatimonadaceae (Gemmatimonadetes; 4.45~6.04%), Micrococcaceae (Actinobacteria; 2.12~4.30%) and Cytophagaceae (Bacteroidetes; 1.32~2.11%) were dominant families (Fig. 3c and supplementary Table S3). The
short-term Ca5 fertilization practices, increased the relative abundance of *Gemmatimonadaceae.*

Figure 2. Beta diversity analysis and Principal Component Analysis (PCA). a, boxplot of the Unweighted UniFrac distance in groups. b, the heatmap of Unweighted UniFrac distance of samples sequenced. c, the PCA scattered
Figure 3. Relative abundance of OTUs of the dominant bacteria at 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. * p < 0.05 vs. CK group. All differences were called by Kruskal-Wallis test.
Figure 4. The stacks and statistical analysis for OTUs’ relative abundance of the dominant genera. a and b, the stacks and statistical analysis for OTUs’ relative abundance of the dominant genera, respectively. * p < 0.05 vs. CK group. All differences were called by Kruskal-Wallis test.
(Gemmatimonadetes family) and Rubrobacteriaceae (Actinobacteria; p<0.05, Figure 3d), but decreased that of the Micrococccaceae (Actinobacteria; p<0.05) in the top 10 families. At the genus level, we identified the abundant genera in groups are Pseudarthrobacter (1.80~3.80%), Sphingomonas (1.08~1.56%), Gaiella (0.96~1.39%), Rubrobacter (0.36~1.02%) and Streptomyces (0.84~1.12%; Figure 4a and supplementary Table S4). We observed that the short-term Ca5 fertilization practice decreased Gemmatimonas (Gemmatimonadetes) abundance (p<0.05, Figure 4b), and Ca4 decreased Pseudarthrobacter(Actinobacteria) and Rubrobacter (Actinobacteria; p<0.05, Figure 4b) in the top 10 genera. LEfSe analysis showed that the family Commononadaceae was dominant bacterium in Ca5 group (Figure 4c); Nitrospira phylum and Nitrospiraceae family were dominant bacteria in Ca1 group (Figure 3b and Figure 4c).

DISCUSSION

We observed that the soil organic matter, available N, P, and K and total N and P as well as the grape quality indicators like tannin and anthocyanin contents were increased by Ca fertilizations. In addition, the soil bacterial community diversity was changed by Ca fertilizer. These findings demonstrated the influence of Ca-fertilizers on soil fertility and grape quality.

It has been reported that the content of soil organic matter, available N, P and K as well as total N and P contents in the experiment plots were very low (Zebire et al., 2019); the Mg content and K/Mg ratio in soil determine the sugar content and grape quality (Bybordi et al., 2010). The K-fertilizers overuse and high available Ca might block the absorbance of Mg, and thus decreased the content of soluble solid and sugar content in grape berry (Bybordi et al., 2010). Our examination revealed that the content of soluble solid in grape berry was declined by Ca fertilization practices and the contents of anthocyanin and total phenol were increased by all fertilization practices. The decreased soluble solid in grape berry might indicate the declined Mg absorbance.

The accumulations of sugar and polyphenols determine wine grape quality and impress the flavor and aroma of wine (Urcan et al., 2016). Phenols, including tannin, polyphenols and flavonoids with anti-hyperglycemic and antioxidiant effect have positive effects on human health (Giovinazzo et al., 2015; Hernándezsalinas et al., 2015; Gross 2016). In addition, tannin controls the free thiols contents in wine and confers wine aroma (Larcher et al., 2015; Capone et al., 2017), while the attractive color of wine is under control of anthocyanin and tannin (Damberg et al., 2006; Nogales-Bueno et al., 2015). The increased content of soil available N, K and P and grapey tannin, anthocyanin, total phenols, and titratable acid by Ca-fertilizers suggested the increased soil fertility and grape quality by Ca-fertilization practices.

Many studies have evaluated the influence of intercropping system and agricultural practices to improve soil cultivability or plant tolerance by regulating the communities of environmental bacteria (Jamieson et al., 2002; Marschner et al., 2003; Yang et al., 2009; Fawaz 2013; Zolla et al., 2013; Ullah et al., 2019). Our present study demonstrated that Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes and Bacteroidetes were the dominant phyla at the phylum level. This composition was inconsistent with the reported soil bacterial structure that Acidobacteria, Actinobacteria and Proteobacteria were the major soil bacteria (Janssen et al., 2002; Spain et al., 2009). In addition, De Brunyn et al., 2011 reported that phylum Gemmatimonadetes are one of the top 10 soil bacterial clusters accounting for 0.2%~6.5% of total soil bacterial community (DeBruyn et al., 2011).

In our study, we confirmed short-term Ca fertilization practices, especially Ca5, decreased the abundance of Gemmatimonadetes, but increased that of the Nitrospira. In addition, we observed that the Ca5 fertilization practices, increased the relative abundance of Gemmatimonadetes and Rubrobacteriaceae, but decreased that of the Micrococccaceae. At the genus level, we observed that the short-term Ca5 fertilization practice decreased Gemmatimonas abundance, and Ca4 decreased Pseudarthrobacter and Rubrobacter. Many of the soil bacteria relate to the tolerance or defense against stresses in rhizosphere (Marschner et al., 2003; DeBruyn et al., 2011; Yandigeri et al., 2012; Ullah et al., 2019), like Actinobacteria and Gemmatimonadaceae (Ullah et al., 2019). De Brunyn et al., in 2011 reported that the relative abundance of Gemmatimonadetes was influenced by the soil drought degree or moisture, N content and organic matter content (DeBruyn et al., 2011), and that in the soil samples for the desert or arid soil were higher than those from the forest or pasture. The dynamic changes of Gemmatimonadetes bacteria with temperature and over time in terrestrial systems implicated that they are important members of soil communities (DeBruyn et al., 2011), and might be related to the plant tolerance to abiotic stresses like drought and hot (Ullah et al., 2019). De Brunyn et al., 2011 also found that the relative abundance of Gemmatimonadetes bacteria were associated with Shannon index of noncrop plants (DeBruyn et al., 2011). Yandigeri et al., suggested that drought-tolerant endophytic actinobacteria Streptomyces coelicolor DE07, S. olivaceus DE10 promoted the growth of wheat (Triticum aestivum) under water stress conditions (Yandigeri et al., 2012). Ullah et al reported that Gemmatimonadaceae were dominant in drought-treated rhizosphere (Ullah et al., 2019). Our present study demonstrated that the decreased abundance of Gemmatimonadetes as well as the contradiction of increased Gemmatimonadaceae division. The decreased Gemmatimonadetes bacteria by Ca-fertilizers might relate with the decreased soil fertility or organic matter content.
Similar changes were found in the Actinobacteria, implicating the different functional division of Gemmatimonadetes and Actinobacteria bacteria in response to the Ca-fertilizers. The changed abundance of Gemmatimonadaceae and Actinobacteria bacteria might correlate with the Ca-fertilizer induced abiotic stresses in rhizosphere.

Nitrospirae phylum and Nitrospiraceae family were dominant bacteria in Ca1 group, indicating the stimulation on them by low Ca level. Nitrospiraceae family members contain chemolithoautotrophic aerobic nitrite-oxidizing bacteria (Nitrospira genus) which are representatives of the predominant known nitrite oxidizers catalyzing the second step of nitrification and are almost ubiquitously distributed in oxic habitats. The increased Nitrospirae phylum abundance by Ca-fertilizers might suggest the enhanced nitrification and N cycling by grape roots, which enhanced the conversion and absorption of N in soil.

**Conclusion:** Here in this study, we demonstrated that the soil biochemistry parameters (including organic matter, available NPK and total NP) as well as the quality indicators (eg. tannin, anthocyanin, total phenols, soluble solid and titratable acid) were influenced by the administration of short-term Ca-fertilizers. The increased soil fertility might suggest Ca-fertilizers were suitable fertilization practices for the alkaline loamy sand. The significant increase in total titratable acid and total phenols in grape berry might suggest the efficacy of Ca-fertilizers on improving the quality of grape. Ca-fertilizers, especially the administration of 323.69 kg/hm² Ca(NO₃)₂·4H₂O (Ca5 fertilization practice) altered the soil bacterial communities, including Gemmatimonadetes and Actinobacteria bacteria, which might be associated with the plant defense or tolerance to abiotic stresses. In addition, these altered bacteria might be the sensitive species to Ca-fertilizers induced environmental changes in rhizosphere soil.

**Conflict of interest:** None.

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**Supplementary files:**
- **Figure S1.** The length distribution of clean tag (a) the rarefaction curves (b) and Shannon-Wiener curves (c) of samples sequenced.
- **Table S1.** The statistics of the tags and OTUs in samples sequenced.
- **Table S2.** The relative abundance of phyla in groups.
- **Table S3.** The relative abundance of families in groups.
- **Table S4.** The relative abundance of genera in groups.

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