



**Full Length Article**

## Effect of Various Mulches on Soil Chemical Properties and Rhizosphere Bacteria of Wine Grape (*Vitis vinifera*)

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### Abstract

Mulching has been used widely to improve the production capacity of the soil, yield and the quality of wine grapes (*Vitis vinifera* L. cv. Cabernet Sauvignon). However, the ecological mechanisms underlying the mulching materials were not well understood. This study aimed to evaluate the effects of different mulching materials on soil chemical properties, bacterial community and wine grape quality. Experiments were performed at a grape-yard on the six-year-old wine grape '*Vitis vinifera* L. cv. Cabernet sauvignon. Five kinds of mulching materials include living turfgrass (Grass), living *Herba portulacae* (Por), inorganic plastic black film (Film), organic chips of wood (Wood), and grape branches (Branch) were applied, while clean tillage (CK) was treated as a control. Soil chemical properties and grape quality indicators were measured. Soil bacterial community diversity was detected using the Illumina Miseq sequencing for the 16S rRNA gene V3-V4 region. Mulching with plastic film, wood chips and *Herba portulacae* (Por) increased the content of soil organic matter, available N, P and K, total N and P. Film, grass, branch and Por mulching materials improved the content of tannin, anthocyanin, total phenol and titratable acid in grape ( $p < 0.05$ ). *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria* and *Gemmatimonadetes* were abundant bacteria. Mulching with grape branch and *portulacae* increased the relative abundance of *Gemmatimonadetes* and *Chloroflexi* phylum, *Gemmatimonadaceae* family, and decreased *Micrococcaceae* family and *Pseudarthrobacter* genus. Mulching with living, organic, or inorganic changed chemical properties and grape quality indicators. These changes might be correlated with the altered soil bacterial community diversity and richness. © 2022 Friends Science Publishers

**Keywords:** Mulching; Soil chemical properties; Wine grape quality; 16S rRNA gene sequencing; Rhizosphere bacteria; *Vitis vinifera* L. cv. Cabernet sauvignon

### Introduction

Mulching is a major practice to reduce soil evaporation, control salinity and increase crop yield (Aragüés *et al.* 2014). Many studies have confirmed the benefits of soil mulching in improving plant growth and yield as well as in modulating the soil physicochemical properties like soil pH, moisture, salinity and sodicity, total porosity, available nitrogen (N) and organic matter (Iles 1999; Aragüés *et al.* 2014; Ni *et al.* 2016; Wang *et al.* 2017; Qu *et al.* 2019). Mulching is also effective in the modulation of soil bacterial community (Qian *et al.* 2015; Farmer *et al.* 2017; Munoz *et al.* 2017).

*Vitis vinifera* is a worldwide cultivated fruit due to the rising position of the wine business in the national economy. The texture and aroma of wine are determined by the varieties, ecological environment, and agricultural practices (Yuyuen *et al.* 2015; Urcan *et al.* 2016; Kok

and Bal 2017; Mencarelli and Bellincontro 2018). The contents of sugar, polyphenols, soluble solids, tannins, phenol compounds and sugar-acidity ratio determine wine's quality and economic value (Yuyuen *et al.* 2015; Mencarelli and Bellincontro 2018). These indicators are variable and robustly influenced by factors like temperature, fertilization, soil management practices, and microbes (Aragüés *et al.* 2014; Leeuw *et al.* 2014; Urcan *et al.* 2016; Kok and Bal 2017; Huang *et al.* 2018). Among these factors, soil microbes play a critical role in regulating processes such as the decomposition of organic matter, nutrient cycling, as well as disease suppression. Especially, rhizosphere bacteria could regulate the crop yield and quality, promote 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). Mulching has been reported to

play an important role in controlling the structure of soil bacterial communities (Farmer *et al.* 2017; Munoz *et al.* 2017). However, the relationships between the mulching materials and bacterial communities in the rhizosphere of grapes were still unclear. Furthermore, the micro-ecological mechanism under the mulches and yield and quality of the grape needs to be demonstrated.

There are many kinds of mulch, divided into organic and inorganic mulch. The organic mulches consist of animal and plant residues. The most commonly used organic mulches include straws, husks, grasses, cover crops (live mulches), saw dust, compost and manures (Iqbal *et al.* 2020). While the most frequently used inorganic mulch throughout the world is polyethylene plastic mulch, which may bring potential environmental pollution (Zhang *et al.* 2021). Mulches can potentially reduce weed infestation and evaporation losses and enhance soil's percolation and retention rate. It was reported that straw mulch could decrease the rate of evaporation by 35% (Iqbal *et al.* 2020). Non-living mulch materials had the greatest capability in moisture conservation in soil compared to un-mulched soil.

We performed this study to investigate the soil mulching-induced changed in soil bacterial community diversity, soil chemical properties and the fruit quality of grape (*V. vinifera* L. cv. Cabernet sauvignon). We hypothesized that different organic mulches have different effects on soil. Illumina Miseq sequencing was performed to determine the structure and richness of soil bacterial communities. Comparative analyses were performed to analyze the different effects of mulching materials on soil bacterial diversity and chemical properties. Our findings would provide a theoretical basis for the high-quality cultivation of wine grape in dry areas.

## Materials and Methods

### Experimental Field Condition

The experimental site was located in the wine grape planting base of Lilan Winery, at the eastern foothills of Helan Mountain, Minning town, Yongning county, Yinchuan city, Ningxia province, China (latitude 38° 16' 38' ' N, longitude 105° 58' 20' ' E, above sea level 1129 m), which is characterized by a temperate arid climate with low rainfall (~200 mm annually), high evaporation (~1580 mm annually), high total solar radiation amount (~6100 MJ/km<sup>2</sup> annually) and short frost-free period (~176 days). The soil chemical properties are shown in Table 1.

### Experimental Materials and Design

Our experiment was conducted at a grape-yard with six-year-old *V. vinifera* L. cv. Cabernet sauvignon wine was planted over two growing seasons from April 2017 to

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

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

The significant differences between the groups are marked by different lowercase letters

October 2018. Grapevines were planted in north-south direction (n = 20 in each line) with 0.6 m × 3.5 m planting space, with a final density of 4760 plants/hm<sup>2</sup>. Thirty experimental plots with 60 grapevines in each plot were randomly divided into five groups (five kinds of mulches, n=6): (1) turf grass (Grass group), (2) *Herba portulacae* (Por group, sowed with 30 kg/hm<sup>2</sup> seeds), (3) plastic black film (Film group, 0.008 mm thickness), (4) wood chips (Wood group, 4-6 cm in length), (5) chips of grape branches (Branch group; 1-2 cm in length). The routine clean tillage (10 cm deep) without mulching was control (CK group). Corresponding materials covered the soil surface (width of 100 cm) under grapevine for all treatments. Grapevines were regularly irrigated with dropper facilities and conventionally fertilized. At the end of October in each year, mulching films were reclaimed and other mulching materials were buried into soil. The experimental plots were divided into 5 groups with 6 repetitions.

### Soil Chemical Parameters Measurement

Root rhizospheric soil samples (5-60 cm in depth) were collected from five randomly selected plants in each plot. Samples were air-dried, ground, filtered and dissolved into distilled water (1: 3(v/v) =soil/water). Soil organic matter (organic carbon) was determined using K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> digestion methods. Available N, P, and K content were determined using alkaline hydrolysis diffusion methods, 0.5 mol/L NaHCO<sub>3</sub>-Mo-Sb colorimetry and 0.5 mol/L NaHCO<sub>3</sub>-flame photometric methods, respectively. Total N and P content were determined using H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> digestion-Nessler's reagent methods and vanadium molybdate yellow colorimetric method, respectively. All detections were performed following the methods as previously described by Bao SD (Bao 2000). Five replications randomly selected from each plot within one group were tested for each experiment.

### Grape Quality Properties Measurement

Twenty grapes were harvested from each plot in September 2018 and were ground into juice. The solid soluble content was detected immediately using a MISCO Palm Abbe™ handheld digital refractometer

(MISCO PA201, Misco, Solon, OH, USA). According to the methods reported by titratable acid, tannin and total phenols content were detected using NaOH titration methods, Folin-Denis assay and Folin-Ciocalteu methods (Li *et al.* 2000). Anthocyanin content was detected using pH-differential spectrophotometry (Li *et al.* 2000). All experiments were performed in 5 replications.

### Soil bacterial DNA extraction and Illumina sequencing

Three Rhizospheric soil samples (5–60 cm in depth) were collected from each plot (250 mg of each plot x 3 repeats). According to the manufacturer's instructions, bacterial DNA samples were extracted from soil samples using a MOBIO PowerSoil DNA Isolation Kit (MO BIO Laboratories, USA). DNA quality was determined using a NanoDrop ND-2100 spectrophotometer (NanoDrop Technologies, USA). PCR amplification was performed using the universal primer pairs (515F/806R with barcode) for the 16S rRNA gene V3 and V4 regions and TransGen AP221-02 TransStart Fastpfu DNA polymerase (TransGen Biotech, China). An equal amount of the DNA samples within one plot were pooled and used to construct a DNA library using a DNA PCR-Free Sample Preparation Kit (Illumina, USA) according to the manufacturer's instructions. Illumina MiSeq platform with a pair-end (PE) 2x150 bp model was employed for the 16S rRNA gene sequencing.

### Data processing and analysis

Sequencing data (Fastq files) were processed using Trimmomatic (Bolger *et al.* 2014), Pear ( $P < 0.0001$ ) (Zhang *et al.* 2013), FLASH (<http://ccb.jhu.edu/software/FLASH/>) and usearch program (Alloui *et al.* 2015) for cleaning the raw data via removing the reads < 50 bp and barcode reads, data splicing and quality filtering, removing Chimera reads, respectively. Tags in short length (< 200 bp) were removed using mothur (Yang *et al.* 2014). Operational taxonomic units (OTUs) with 97% identity were identified and clustered using Uparse software (Edgar 2013) and single OTUs were removed. Rarefaction curves, Shannon-Wiener curves, and the species accumulation curves of the samples were presented using mothur (Yang *et al.* 2014). The alpha (Chao1, observed OTUs, PD whole tree and Shannon) of and beta diversity index (Unweighted UniFrac distance) of each sample was calculated and compared between groups. For the annotation of OTUs, Ribosomal Database Project (RDP) classifier program (Cole *et al.* 2008) and the SILVA ribosomal RNA (rRNA) database (Quast *et al.* 2012) were used. Principal Component Analysis (PCA) was performed for sample clustering. The relative abundances of OTUs at each taxonomic level were calculated and

different taxonomies among groups were identified using Kruskal-Wallis's test with the threshold of  $P < 0.05$ . The cluster analysis tree was built using the genetic distance UPGMA (Unweighted pair group method with arithmetic mean) algorithm (Dongen and Winnepeninckx 1996).

### Statistical analysis

All data of chemical properties and grape quality parameters were expressed as the mean  $\pm$  standard deviation (SD). Analysis of variance was performed using SPSS 21.0 and multiple comparisons were performed using the LSD method ( $\alpha = 0.05$ )  $P < 0.05$  was considered as significant difference.

## Results

### Mulching methods effect on soil chemical properties and wine grape quality

This study showed that mulching with plastic film, grass, wood chips, and herba portulacae (Por) increased the contents of soil organic matter and available N significantly ( $P < 0.05$ ). But mulching with grapevine branch decreased soil organic matter, available N and P compared with CK and other mulching materials ( $P < 0.05$ , Table 2). Grass mulching decreased soil available P and K (significantly,  $P < 0.05$ ) and total N versus CK (insignificantly,  $P > 0.05$ , Table 2). Mulching increased tannin, anthocyanin, total phenols and titratable acid but decreased soluble solid contents in wine grape ( $P < 0.05$ , Table 3). These results suggested that plastic film and organic mulching materials like wood and portulacae were efficacious in improving soil fertility and might be recommendable agricultural practices for improving the soil cultivability. Evidently, mulching materials like wood significantly increased the Total N (increased by 63%) and Total P (increased by 21%). Por significantly increased the OM and Available N by 42 and 85%, respectively. The use of grass and grapevine branches for mulching showed uncertain efficacies in decreasing the soil cultivability.

### General analysis of the Illumina 16s rRNA gene sequencing data

To investigate the effect of mulching on the modulation of edaphology, Illumina 16S rRNA sequencing was performed to detect the bacterial community diversity in rhizosphere soil. Illumina Miseq sequencing generated 1,550,850 raw tags, including 536,970 clean tags (Table S1). Most (98.76%) of these tags were in the length of 400~440 bp (Fig. 1a). In total 78,182 OTUs were identified, with an average number of 2,606 tags per sample (Table S2). The rarefaction curves of samples sequenced showed that higher numbers of OTUs might

**Table 2:** Mulching methods effect on soil chemical properties in maturity stage

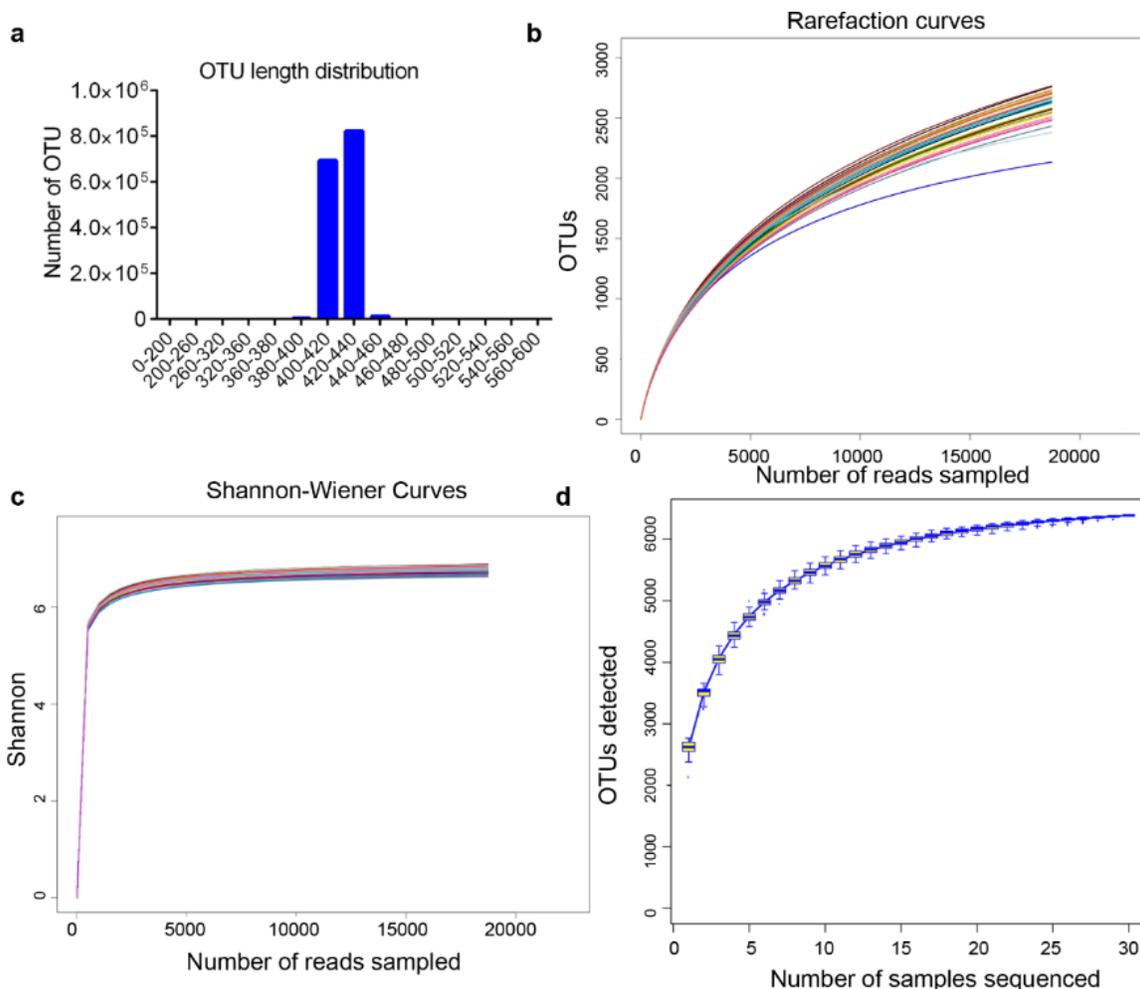
Group	Organic matter (g/kg)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	Total N (g/kg)	Total P (g/kg)
CK	7.27 ± 0.22 <sup>c</sup>	25.59 ± 0.13 <sup>d</sup>	6.63 ± 0.12 <sup>c</sup>	217.20 ± 0.58 <sup>cd</sup>	0.46 ± 0.00 <sup>c</sup>	0.28 ± 0.01 <sup>b</sup>
Film	9.73 ± 0.15 <sup>b</sup>	40.18 ± 2.20 <sup>b</sup>	18.14 ± 0.55 <sup>a</sup>	311.63 ± 1.60 <sup>a</sup>	0.59 ± 0.00 <sup>d</sup>	0.31 ± 0.01 <sup>ab</sup>
Grass	7.58 ± 0.08 <sup>c</sup>	32.96 ± 0.56 <sup>c</sup>	5.12 ± 0.25 <sup>d</sup>	208.33 ± 0.45 <sup>d</sup>	0.39 ± 0.01 <sup>f</sup>	0.35 ± 0.03 <sup>a</sup>
Wood	9.62 ± 0.06 <sup>b</sup>	34.23 ± 1.49 <sup>c</sup>	5.10 ± 0.22 <sup>d</sup>	313.89 ± 11.07 <sup>a</sup>	0.75 ± 0.01 <sup>a</sup>	0.34 ± 0.01 <sup>a</sup>
Branch	5.59 ± 0.01 <sup>d</sup>	24.21 ± 0.39 <sup>d</sup>	3.38 ± 0.21 <sup>e</sup>	224.00 ± 3.03 <sup>c</sup>	0.66 ± 0.00 <sup>c</sup>	0.29 ± 0.00 <sup>b</sup>
Por	10.32 ± 0.06 <sup>a</sup>	47.46 ± 0.65 <sup>a</sup>	11.74 ± 0.23 <sup>b</sup>	289.60 ± 0.24 <sup>b</sup>	0.69 ± 0.01 <sup>b</sup>	0.31 ± 0.01 <sup>ab</sup>

The significant differences between the groups are marked by different lowercase letters. Por, herba portulacae

**Table 3:** Mulching methods effect on the quality of wine grape berry

Group	Tannin (mg/kg)	Anthocyanin (mg/kg)	Total phenols (mg/kg)	Soluble solid (%)	Titrateable acid (%)
CK	13.81 ± 0.22 <sup>c</sup>	5.71 ± 0.03 <sup>c</sup>	16.02 ± 0.21 <sup>c</sup>	25.56 ± 0.14 <sup>a</sup>	0.62 ± 0.01 <sup>b</sup>
Film	17.30 ± 0.41 <sup>a</sup>	7.29 ± 0.01 <sup>c</sup>	20.06 ± 0.55 <sup>a</sup>	23.24 ± 0.33 <sup>c</sup>	0.70 ± 0.01 <sup>a</sup>
Grass	16.62 ± 0.37 <sup>a</sup>	7.58 ± 0.05 <sup>c</sup>	20.29 ± 0.25 <sup>a</sup>	25.22 ± 0.45 <sup>a</sup>	0.71 ± 0.01 <sup>a</sup>
Wood	12.52 ± 0.14 <sup>d</sup>	8.49 ± 0.19 <sup>a</sup>	18.98 ± 0.22 <sup>b</sup>	25.24 ± 11.07 <sup>a</sup>	0.65 ± 0.01 <sup>b</sup>
Branch	14.73 ± 0.19 <sup>b</sup>	6.63 ± 0.08 <sup>d</sup>	16.70 ± 0.21 <sup>c</sup>	24.48 ± 3.03 <sup>ab</sup>	0.73 ± 0.01 <sup>a</sup>
Por	17.25 ± 0.26 <sup>a</sup>	8.06 ± 0.23 <sup>b</sup>	19.93 ± 0.23 <sup>a</sup>	23.80 ± 0.24 <sup>bc</sup>	0.72 ± 0.01 <sup>a</sup>

The significant differences ( $P < 0.05$ ) between the groups are marked by different lowercase letters. Por, herba portulacae



**Fig. 1:** The OTUs distribution and curves of samples sequenced. (a) the length distribution of the OTUs, (b) the rarefaction curves of the samples showing the depth of sequencing and the possibility of OTUs numbers, (c) the Shannon curves showing the depth of sequencing and the possibility of bacterial diversity, (d) Specaccum species cumulative curve showing the increase rete of new species with sequencing size

be produced with deeper sequencing (Fig. 1b). Shannon-Wiener curves showed that deeper sequencing would not increase the bacterial diversity and the present Illumina sequencing data was sufficient for diversity analysis (Fig. 1c). Species accumulation curves revealed that the sample size was sufficient to reflect the richness of the community (Fig. 1d). Further analysis showed there were no differences in the alpha diversity indicators (e.g., Chao1, observed OTUs, PD whole tree and Shannon) among groups (Fig. 2a–d) and the unweighted\_unifrac\_distance in each group (Fig. 3a). PCA showed that samples in the Wood, Grass and Por groups were not discriminant, while the clusters of other samples in the CK, Film and Branch groups were relatively compact (Fig. 3b). Based on the annotation and abundance calculation of OTUs, we observed some OTUs or bacteria were differentially distributed in the samples (Fig. 4). Accordingly, we identified the differential rhizosphere bacteria in response to the mulching methods.

#### Identification of the differential bacteria by different mulching materials

*Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes* and *Bacteroidetes* were abundant in all groups (4.46–24.28%; Fig. 5a and supplementary Table S3). *Gemmatimonadaceae* (*Gemmatimonadetes*), *Micrococcaceae* (*Actinobacteria*), *Anaerolineaceae* (*Chloroflexi*) and *Cytophagaceae* (*Bacteroidetes*) were abundant bacteria at the family level (Fig. 5b and supplementary Table S4). The relative abundance of *Gemmatimonadetes* phylum and *Gemmatimonadaceae* family was increased by mulching grape branches (from  $8.77 \pm 0.37$  to  $10.46 \pm 0.18\%$   $P < 0.05$  and from  $4.44 \pm 0.21$  to  $7.04 \pm 0.75\%$ ,  $P < 0.05$ , respectively) and herba portulacae (from  $8.77 \pm 0.37$  to  $9.91 \pm 0.67\%$ ,  $P > 0.05$ , from  $4.44 \pm 0.21$  to  $5.83 \pm 0.65\%$ ,  $P < 0.05$ , respectively; Fig. 5c and d). Grass mulching decreased *Gemmatimonadetes* phylum (from  $8.77 \pm 0.37$  to  $6.88 \pm 0.30\%$ ,  $P < 0.05$ ) and *Micrococcaceae* family (from  $4.33 \pm 0.47$  to  $3.47 \pm 0.08\%$ ,  $P < 0.05$ , Fig. 5d). The abundant genus *Pseudarthrobacter* (*Actinobacteria*,  $3.79 \pm 0.97\%$ ) were decreased in branch ( $1.34 \pm 0.79\%$ ,  $P < 0.05$ ) and Por group ( $1.31 \pm 1.24\%$ ,  $P < 0.05$ ) and abundant genus *Sphingomonas* (*Proteobacteria*,  $1.54 \pm 0.20\%$ ) were decreased in Film ( $0.91 \pm 0.22\%$ ,  $P < 0.05$ ) and Grass group ( $1.06 \pm 0.04\%$ ,  $P < 0.05$ ; Fig. 6a–b and supplementary Table S5).

#### Discussion

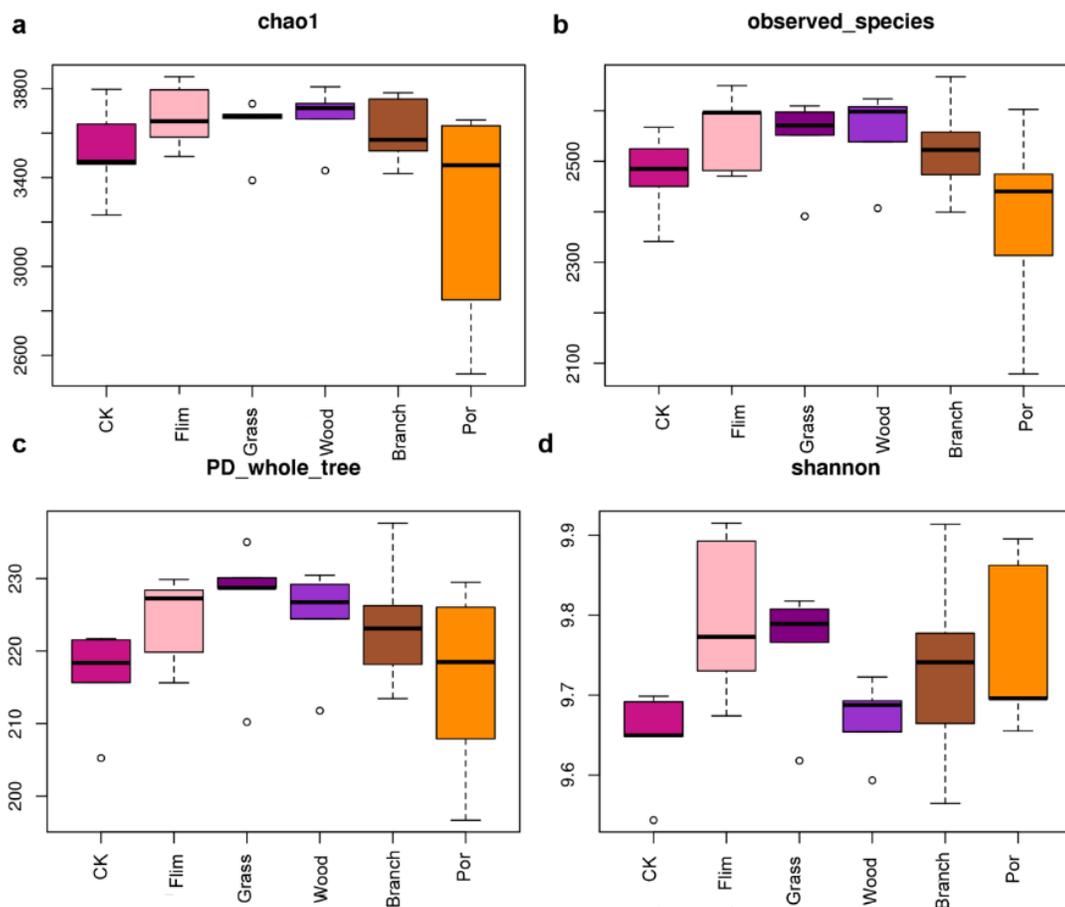
Soil mulching could improve the soil chemical parameters (including organic matter, available NPK and total NP). Materials of grape branches and herba

portulacae changed the community structure of soil bacteria. Mulching materials showed multiple benefits in plant growth via regulating soil temperature, moisture, total porosity and organic matter, and decreasing soil evaporation. The efficient effect of mulching materials on ecological restoration and the soil physicochemical properties showed mulching had important roles in regulating and modulating the edaphology. It has been reported that the soil bacterial diversity could be altered by the soil physicochemical properties (Farmer *et al.* 2017). Our study found that mulching could improve the soil chemical parameters, such as organic matter, available NPK and total NP. Hence, we confirmed the effect of mulching on soil physicochemical properties and the influence of mulching on soil bacteria community diversity.

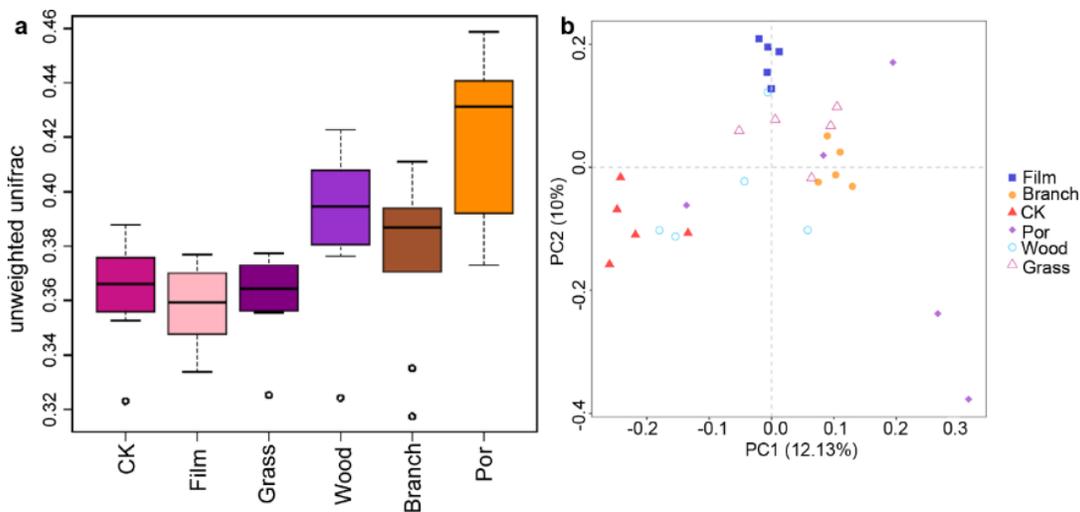
It has been reported that mulching with wood chips significantly promoted the growth of plant as well as improved the available N and organic matter in soil, mulching with organic materials (like green waste compost and pine bark) for a long-term (2-years) increased the soil organic matter, total N, mineral N and available P and K, while mulching with turf grass only increased the soil total N and available K, suggesting the weak contribution of grass mulching to soil fertility. These results in the above reports were inconsistent with the results in our studies that grass and branch materials showed questionable effects on soil fertility. In this present study, mulching significantly increased the soil chemical properties. We speculate that these differences may be due to the differences in depth and soil type (Duryea *et al.* 1999; Iiles 1999; Ni *et al.* 2016). Also, other reports showed that the organic mulches significantly decreased the pH value in soil of fine sandy loam (Billeaud and Zajicek 1989; Duryea *et al.* 1999; Wang *et al.* 2017), whereas some showed opposite opinions (Iiles 1999; Ni *et al.* 2016). Despite the aforementioned differences, our present study confirmed that mulching with plastic film and herba portulacae had high efficacies in improving the fertility of mixed soil samples.

Mulching showed considerable efficacy in plant growth and crop quality (Qu *et al.* 2019). Ni *et al.* (2016) reported that mulching with different materials improved plant height, root activity, electric conductivity and the content of chlorophyll a/b, water, soluble sugar and proline in leaves. However, revealed that mulching did not significantly change the growth and height of *Sophora japonica*. Here in our present study, mulching improved grape quality indicators (e.g., tannin, anthocyanin, total phenols and titratable acid) but decreased soluble solid contents in wine grape.

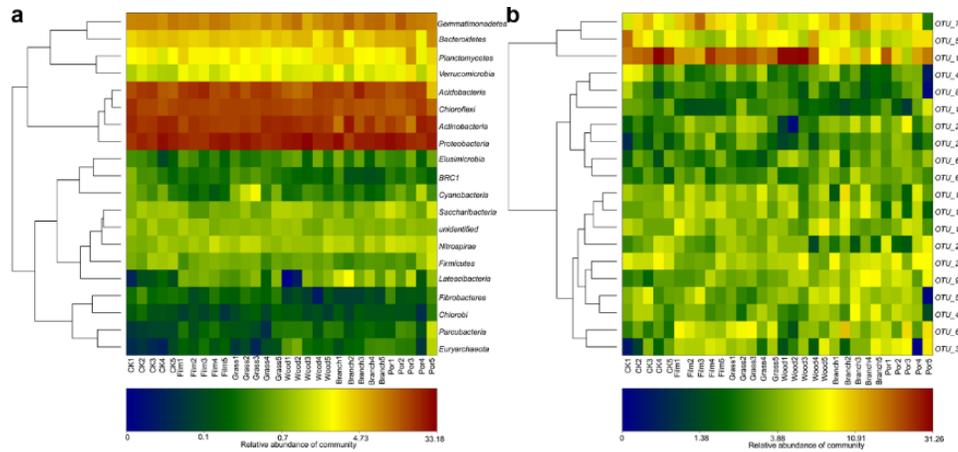
Mulching-induced changes in soil physicochemical properties may significantly influence bacterial diversity or richness (Aragüés *et al.* 2014; Farmer *et al.* 2017). The soil bacterial community composition here was accorded



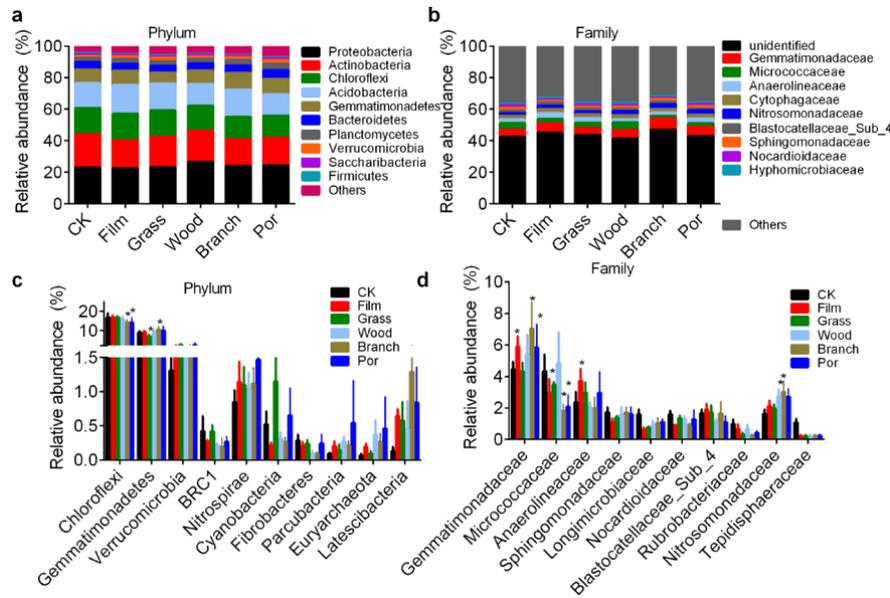
**Fig. 2:** The alpha diversity indicators. Difference in alpha diversity indicators including Chao1 (a), observed OTUs (b), PD whole tree (c) and Shannon (d) is analyzed by Kruskal-Wallis's test. Grass, soil surface was mulched with natural grass (< 5 cm); Por, herba portulacae; Film, black plastic film; Wood, wood chips; Branch, chips of dry grape branches; CK, with nothing but clean tillage



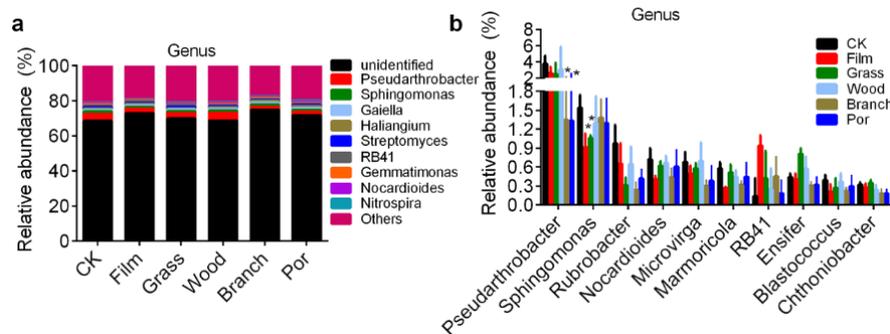
**Fig. 3:** Beta diversity analysis and Principal Component Analysis (PCA). (a) boxplot of the Unweighted UniFrac distance in groups, (b) the PCA scattered plots of samples sequenced



**Fig. 4:** The heatmaps of the relative abundance of the top 20 phylum (a) and top 20 OTUs (b). Red indicates high relative abundance, and blue notes low relative abundance of the related bacterial phylum (a) or OTUs (b) in each sample sequenced



**Fig. 5:** Relative abundance of OTUs of the dominant bacteria at phylum and family level. (a) and (b) the stacks of OTUs' relative abundance of the dominant phyla and family, respectively. (c) and (d) the statistical analysis for OTUs' relative abundance of the top 10 phyla and family, respectively. \*  $P < 0.05$  vs. CK group. All differences were called by Kruskal-Walli's test



**Fig. 6:** The stacks (a) and statistical analysis (b) for OTUs' relative abundance of the dominant genera. \*  $P < 0.05$  vs. CK group. All differences were called by Kruskal-Walli's test

with the reported fact that *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes* and *Bacteroidetes* were dominant soil bacteria (Janssen *et al.* 2002; Spain *et al.* 2009; Davis *et al.* 2011; Miyashita 2015). Detected that the abundance of *Chloroflexi* and the soil enzyme activity which linked to the organic matter decomposition (*e.g.*,  $\beta$ -glucosidase,  $\beta$ -D-cellobiosidase, Phosphatase and N-acetyl- $\beta$ -D-glucosaminidase) were increased in soil at the same time (Delgado-Baquerizo *et al.* 2018). This present study found that the relative abundance of *Chloroflexi* phylum was decreased by mulching with the chips of grape branches and herba portulacae.

We speculated that there might be a positive association between the *Chloroflexi* bacteria and soil organic matter decomposition. Here in our present study, we demonstrated that the relative abundance of *Chloroflexi* phylum was decreased by mulching with the chips of grape branches and herba portulacae. The decreased *Chloroflexi* phylum was not in line with the soil organic matter content in the two groups, suggesting there might not have a direct link between *Chloroflexi* phylum abundance and organic matter decomposition.

Many of the soil bacteria, like *Actinobacteria* and *Gemmatimonadaceae*, are related to the tolerance or defense against stresses (Marschner *et al.* 2003; DeBruyn *et al.* 2011; Yandigeri *et al.* 2012; Ullah *et al.* 2019). For instance, the dynamic changes of *Gemmatimonadetes* with temperature and time in terrestrial systems implicated that the crucial role of these environmental factors in soil ecology systems (DeBruyn *et al.* 2011). Accordingly, much effort has been performed to evaluate the influence of agricultural practices on improving soil cultivability or plant tolerance by regulating the communities of bacteria (Jamieson *et al.* 2002; Marschner *et al.* 2003; Yang *et al.* 2009; Fawaz 2013; Zolla *et al.* 2013; Ullah *et al.* 2019). Phylum *Gemmatimonadetes* is one of the top 10 soil bacteria (DeBruyn *et al.* 2011). Some studies have shown that the abundant *Gemmatimonadetes* phylum could be influenced by soil organic matter content, drought degree, and N content (DeBruyn *et al.* 2011). Ullah *et al.* (2019) reported that *Gemmatimonadaceae* was dominant in the drought-treated rhizosphere. DeBruyn *et al.* (2011) reported that the abundance of *Gemmatimonadetes* in a desert or arid soil were higher than those from the forest or pasture. The increased abundance of *Gemmatimonadaceae* might be related to the plant tolerance to abiotic stresses like drought and heat (Ullah *et al.* 2019). In our present study, we found that the relative abundances of *Gemmatimonadetes* phylum and *Gemmatimonadaceae* family were increased by branch and herba portulacae mulching. It has been reported that the soil moisture and the soil temperature could be improved by soil mulching (Ni *et al.* 2016; Gu *et al.* 2017; Tan *et al.* 2017; Wang *et al.* 2017; Qu *et al.* 2019).

Accordingly, we assumed that the increased *Gemmatimonadetes* and *Gemmatimonadaceae* here might not be induced by the drought, but by the increased temperature.

At last, we found the two genera *Pseudarthrobacter* and *Sphingomonas* were influenced by mulching. *Pseudarthrobacter* and *Sphingomonas* belongs to the subdivision of *Proteobacteria* and *Actinobacteria*, respectively and the latter was dominant soil bacteria with relative constant abundances in diverse soil types (Janssen *et al.* 2002; Spain *et al.* 2009; Davis *et al.* 2011; Miyashita 2015). This was in consistent with the fact that the *Mircrococcaceae* family was decreased in branch and Por group versus control. These results indicated the important roles of these bacteria in soil ecology and in the growth, development and defense of plants.

## Conclusion

Mulching could improve the soil chemical parameters (including organic matter, available NPK and total NP). However, mulching with inorganic (black plastic film), organic materials (wood chips and chips of grape branches) and living (turf grass and herba portulacae) all improved the contents of tannin, anthocyanin, total phenol and titratable acid in wine grape, but decreased soluble solid content. Soil mulching materials or the chips of grape branches and herba portulacae changed soil bacteria's community structure, including increased *Gemmatimonadetes* phylum, *Chloroflexi* phylum and *Gemmatimonadaceae* family, which were reported to be associated with the plant defense or tolerance to abiotic stresses. The altered abundance of these bacteria indicated the improvement in the resistance to abiotic stresses in plants by mulching materials.

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## Author Contributions

PJ and RW: proposed the research and finalizing the manuscript, QS and JZ: data collection, PJ and RW: DNA analysis and drafted the manuscript. All authors provided critical feedback and helped to shape the manuscript.

## Conflict of Interest

All authors declared there were no conflicts of interest involved.

**Ethics Approval**

Not applicable

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