Fungal diversity rather than bacterial diversity drives the ecosystem multifunctionality of vineyards in a semi-arid region

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Abstract: The presence of multiple ecosystem functions and services (i.e., ecosystem multifunctionality) has been proven to be maintained by biodiversity in natural terrestrial ecosystems. However, the mechanisms by which microbial diversity drives ecosystem functions in vineyards and the effects of ecosystem functions on wine quality remain unknown. Here, fifteen vineyards from five wine sub-regions (Shizuishan, Yinchuan, Yuquanxian, Qintongxian, and Hongshu) in Ningxia were selected to assess the microbial community structure, ecosystem multifunctionality, and wine quality. Overall, each index differed among the vineyards from these five wine sub-regions in Ningxia. High-throughput sequencing revealed that bacterial and fungal communities varied among these vineyards. Bacterial communities were dominated by Actinobacteria, Proteobacteria, Chloroflexi, and Acidobacteria. Ascomycota was the dominant fungal phylum, followed by Basidimycota and Mortierellomycota. In addition, fungal Shannon diversity rather than bacterial Shannon diversity showed a positive relationship with ecosystem multifunctionality. Correlation analysis revealed that ecosystem multifunctionality was positively correlated with wine acidity and negatively correlated with pH value and residual sugar content of wine. Soil chemical functions exhibited relationships with wine quality being similar to those of ecosystem multifunctionality; i.e., positively related to wine acidity but negatively related to wine pH and residual sugar content. However, soil physical functions were negatively correlated with the alcohol and anthocyanin content of wine. The research results show that the ecosystem functions maintained by fungal diversity could be attributed to wine quality of vineyards.

Keywords: microbial diversity, multifunctionality, terroir, wine quality, wine sub-regions
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1 Introduction

Multiple ecosystem functions and services (i.e., multifunctionality) as opposed to a single ecosystem function, such as net primary productivity or nutrient cycling, reflect the combinations of functions of terrestrial ecosystems and the tradeoffs among them¹⁻⁴. Biodiversity plays a vital role in maintaining ecosystem functions. Previous studies have indicated that high diversity of organisms at each trophic level in a food web participating in biogeochemical cycling and energy flow promote the existence of multiple ecosystem functions⁵⁻⁸. Some investigations have found plant diversity to be positively related to ecosystem multifunctionality in natural terrestrial ecosystems⁹⁻¹⁰. Soil microbes, playing vital roles in nutrient cycling and the formation of soil structure in terrestrial ecosystems, have been reported to promote multiple functions in terrestrial ecosystems¹¹⁻¹³. However, agroecosystems manipulated by humans show characteristics that differ from those of natural ecosystems, such as monoculture, meaning low diversity of plants and products exported from agroecosystems. Practices such as fertilization and irrigation conducted in agroecosystems also disturb microbial community structure and diversity. A recent study conducted in farmland revealed that long-term organic fertilizer improved soil multifunctionality by increasing bacterial and fungal diversity. However, none of these studies have focused on how soil microbial diversity would drive multifunctionality of the wine sub-regions in northwest China.

Suitable soil conditions can meet the requirements of fruit-producing plants for water, nutrients, and temperature and thus improve the yield and quality of fruits. Soil nutrient contents...
are the most important factors affecting grape growth and quality. Moreover, grape berry quality determines the quality of the resulting wine and is affected by soil conditions. For example, soil rich in organic matter leads to darker grape skin and higher tannin contents\(^\mathrm{[18,19]}\). Soil rich in phosphorus (P) is helpful for the synthesis of anthocyanins in grape skin\(^\mathrm{[14]}\). Some studies have also shown that macronutrients and micronutrients can promote the quality of grape berries and the resulting wine; for instance, higher content of potassium (K) in soil is beneficial for increasing the contents of total phenols and tannins in grapes\(^\mathrm{[15,16]}\). Wine quality differs when grapes are harvested in different regions based on soil water content\(^\mathrm{[17]}\). In vineyards of semiarid and arid regions, soil water holding capacity affected by soil texture has thus been found to influence vine water status and growth, yield, and wine quality\(^\mathrm{[17]}\). Therefore, aspects of soil multifunctionality, including soil structure, nutrient contents, pH and other internal factors of the soil itself, affect the growth and development of berries and thus the quality of grape and wine\(^\mathrm{[18,19]}\). In addition, photosynthesis, which is affected by chlorophyll, directly influences grape formation, yield, and quality\(^\mathrm{[20]}\). Sucrose, the product of photosynthesis, is transported through vines and stored in grape berries, thus reflecting the influence of photosynthesis on wine quality. Therefore, exploring the relationship between ecosystem multifunctionality and wine quality is of great significance for accurate vineyard management, improving wine quality and increasing farmer income.

The wine industry has developed rapidly in Ningxia of China in recent years. This region is a representative wine grape-producing area in China and enjoys a high reputation globally. At present, the area planted in wine grapes has reached 38,000 hm\(^2\), and the annual production of wine is 130 million bottles, which has supported 120,000 ecological immigrants. *Vitis vinifera* L. cv. Cabernet Sauvignon is the main commercial wine grape variety in Ningxia\(^\mathrm{[21]}\). Overall, the different qualities of wine from different wine sub-regions in Ningxia are caused by external factors, such as soil conditions. However, knowledge about the relationships between microbial diversity and ecosystem multifunctionality of vineyards remains lacking, and the mechanisms by which ecosystem multifunctionality affects the quality of grape berries and the resulting wine remain to be explored. Herein, it was hypothesized that: 1) microbial community diversity and composition, ecosystem multifunctionality and wine quality vary among vineyards from different locations; 2) bacterial and fungal diversity drive ecosystem multifunctionality of these vineyards; and 3) changes in the quality of wine from these vineyards can be attributed to ecosystem multifunctionality.

## 2 Materials and methods

### 2.1 Site description

The experiment was conducted at the eastern foot of Helan Mountain in the Ningxia Autonomous Region of China. Five main wine sub-regions in this area were selected (Shizuishan, Yinchuan, Yuyuanying, Qingtongxia, and Hongsipu) for investigation of the grape cultivar *Vitis vinifera* L. cv. Cabernet Sauvignon. The soil is montane grey-cinnamon soil. The climate is semiarid, with a mean annual precipitation of 225 mm and a mean annual temperature of 9.3°C.

### 2.2 Experimental design and sampling

Fifteen vineyards were chosen from the five wine sub-regions. Two vineyards, named Xiyuwangguan (XYWQ) and Hedong (HED), are in the Shizuishan region. Three vineyards, named Guanlan (GL), Helu (HL), and Zhangyu (ZY), are in Yinchuan region. Four vineyards, named Lilan (LL), Xixiawang (XXW), Baolelijia (BLLJ), and Bagesi (BGS), are in Yuyuanying region. Three vineyards, named Yuhuang (YH), Yuma (YM), and Xige (XG), are in Qingtongxia region. Three vineyards, named Huida (HD), Hongfenjiarong (HFJR), and Luoshan (LS), are in Hongsipu region (Figure 1). Three sites were sampled from each vineyard, with at least 5 m distance between any two sites. The soil and vines were sampled during August 2019. Soil cores from three randomly selected points at each site were sampled from the 0-20 cm layers and combined to form composite samples. After removing stones and roots manually, all fresh soil samples were sieved through a 2 mm mesh. The sieved soil samples were separated into two subsamples, with one subsample being air-dried and then sieved through a 0.25 mm mesh to determine soil chemical and physical properties. Another subsample stored at −80°C was used for high-throughput sequencing. Fifteen kilograms of grapes at commercial maturation were randomly harvested from each vineyard for berry quality determination and for making wine in small (20 L) glass containers.

### 2.3 Soil variable analyses

The soil organic carbon (SOC) content was determined using dichromate oxidation. Soil total nitrogen (TN) content was determined using an automatic Kjeldahl instrument (Kjeltec 8400, FOSS Corporation, Denmark)\(^\mathrm{[22]}\). Soil total phosphorus (TP) content was measured colorimetrically after being digested with H\(_2\)SO\(_4\) and HClO\(_4\). Soil total potassium (TK) content was measured by the NaOH fusion method with atomic absorption spectroscopy (GGK-830, Haiguang Instrument Co., Beijing, China). Soil available phosphorus (aP) content was determined using the Olsen method. The contents of nitrate nitrogen (NO\(_3^-\)-N) and ammonium nitrogen (NH\(_4^+\)-N) extracted by 2 mol/L KCl were determined with a continuous-flow autoanalyzer (Alpkem, OI Analytical, USA), and soil available potassium (aK) content was determined by flame photometry. The soil pH was determined.
using a soil-to-water ratio of 1:2.5. The soil moisture was determined by the cutting ring method. Detailed data on the soil physicochemical properties are shown in Table 1.

<table>
<thead>
<tr>
<th>Vineyard</th>
<th>SOC / g kg⁻¹</th>
<th>TN / mg kg⁻¹</th>
<th>TP / mg kg⁻¹</th>
<th>TK / mg kg⁻¹</th>
<th>NO₃ / mg kg⁻¹</th>
<th>NH₄⁺ / mg kg⁻¹</th>
<th>aP / mg kg⁻¹</th>
<th>aK / mg kg⁻¹</th>
<th>pH</th>
<th>Soil water content %</th>
<th>Soil bulk density / g cm⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYWQ</td>
<td>7.42±0.10</td>
<td>578.07±98.44</td>
<td>116.16±25.25</td>
<td>67.97±3.00</td>
<td>75.43±4.00</td>
<td>1.79±0.75</td>
<td>3.55±1.29</td>
<td>304±30.12</td>
<td>7.43±0.20</td>
<td>7.50±1.00</td>
<td>1.61±0.08</td>
</tr>
<tr>
<td>HED</td>
<td>3.88±1.22</td>
<td>165.82±156.54</td>
<td>184.34±10.74</td>
<td>68.10±2.80</td>
<td>20.68±0.81</td>
<td>0.80±0.10</td>
<td>29.71±5.79</td>
<td>408±33.92</td>
<td>8.18±0.32</td>
<td>10.19±0.65</td>
<td>1.66±0.12</td>
</tr>
<tr>
<td>GL</td>
<td>6.67±0.49</td>
<td>519.68±87.54</td>
<td>102.20±10.32</td>
<td>38.30±1.20</td>
<td>18.77±6.44</td>
<td>0.92±0.27</td>
<td>8.25±1.58</td>
<td>211.88±9.66</td>
<td>8.10±0.11</td>
<td>4.27±0.84</td>
<td>1.54±0.03</td>
</tr>
<tr>
<td>HL</td>
<td>10.10±0.29</td>
<td>666.10±72.03</td>
<td>162.01±10.74</td>
<td>14.83±1.70</td>
<td>11.2±0.06</td>
<td>1.02±0.15</td>
<td>59.68±1.87</td>
<td>217.00±41.36</td>
<td>8.51±0.02</td>
<td>7.02±0.28</td>
<td>1.55±0.17</td>
</tr>
<tr>
<td>ZY</td>
<td>7.95±0.93</td>
<td>787.94±133.36</td>
<td>167.05±32.28</td>
<td>55.63±5.54</td>
<td>22.43±3.23</td>
<td>6.45±0.64</td>
<td>28.61±24.61</td>
<td>547.17±37.03</td>
<td>8.32±0.20</td>
<td>6.20±0.07</td>
<td>1.71±0.07</td>
</tr>
<tr>
<td>LL</td>
<td>4.04±0.45</td>
<td>149.17±10.26</td>
<td>90.61±1.87</td>
<td>25.37±1.56</td>
<td>25.44±1.15</td>
<td>0.64±0.20</td>
<td>10.62±0.65</td>
<td>507.83±19.53</td>
<td>7.96±0.14</td>
<td>3.86±0.39</td>
<td>1.72±0.05</td>
</tr>
<tr>
<td>XW</td>
<td>3.94±0.67</td>
<td>376.45±44.95</td>
<td>83.62±18.29</td>
<td>98.77±1.10</td>
<td>4.76±0.04</td>
<td>0.61±0.09</td>
<td>16.84±3.93</td>
<td>122.50±21.28</td>
<td>8.50±0.26</td>
<td>1.55±0.19</td>
<td>1.55±0.04</td>
</tr>
</tbody>
</table>

Note: XYWQ: Xiyuwangquan; HED: Hedong; GL: Guanlan; HL: Helu; ZY: Zhangyu; LL: Lilan; XW: Xixiawang; BLLJ: Baolelijia; BGS: Bagesi; Y: Yabang; YM: Yuma; XG: Xige; HD: Huida; HIFR: Hongfengjiang; LS: Luoshan. SOC: soil organic carbon. TN: total nitrogen. TP: total phosphorus. TP: total potassium. aP: available phosphorus. aK: available potassium. Different lower-case letters indicate significant differences according to Duncan’s multiple range test at the p<0.05 level. Results are reported as the mean ± SD (n = 3).

2.4 Chemical variation of grapes berries and resulting wine

The sugar content, titratable acidity and pH of grape and wine were determined according to Wang et al. methods[24]. The content of total phenols in wine was determined by the Folin-Ciocalteu method. The wine tannin content was measured by the Folin-Dennis method. The total anthocyanin content of resulting wine was determined by the pH differential method. The total phenolic content was determined with NaNO₂/AICl₃[25]. Flavan-3-ol content was analyzed by the method described by a previous protocol[26].

2.5 Bacterial and fungal throughout grape and sequence processing

Microbial DNA was extracted from soil using the OMEGA Soil DNA Kit (M5635-02) (Omega Bio-Tek, Norcross, GA, USA). The bacterial V3-V4 hypervariable 16S rRNA region and the fungal ITS1 region were amplified by polymerase chain reaction (PCR) using primers. The 338F (5'-AUCTCTACGAGGA GCACGCAG-3') and 806R (5'-GGACTACHVGGTTWTCTAAT-3') primers were designed for V3-V4 fungi. The fungal ITS1 region was amplified by primers I5SSF (5'-GGATAATTAAGTCTGAA CAAGG-3') and I5SIR (5'-GTCGGCTCTCATCGATGCG-3'). The volumes of PCR amplification contained 5 mL of buffer (5x), 0.25 µL of Fast Pfu DNA Polymerase (5 U/µL), 2 µL (2.5 mmol/L) dNTPs, 1 µL (10 mmol/L) of each forward and reverse primer, 1 µL of DNA template, and 14.75 µL ddH₂O. PCR amplification for both bacteria and fungi was performed as follows: 98°C for 5 min; 30 cycles of 98°C for 30 s, 52°C and 55°C for 45 s (bacteria and fungi, respectively), 72°C for 45 s; with a final extension at 72°C for 5 min. Next, the PCR products were verified by 2% agarose gel electrophoresis. The PCR products were mixed in equal density ratios and then purified using a QiaGen Gel Extraction Kit (Qiagen, Germany). The purified amplicons were then sequenced on an Illumina NovaSeqPE250 platform by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

Sequences were filtered and chimera-checked using Quantitative Insights Into Microbial Ecology (QIME)[27]. After chimeric sequences were identified and removed to obtain effective tags, the remaining sequences were clustered by UCLUST and assigned to operational taxonomic units (OTUs) with 97% similarity. The bacteria were identified using the Silva reference database (http://www.arb-silva.de) with the RDP classifier, and the fungi were identified using the Unite database (https://unite.ut.ee/) with the BLAST tool in QIME (http://qiime.org/index.html). Community diversity indices, including rarefaction curves, observed species, the Shannon-Wiener index, and the Chaol estimator were calculated for bacteria and fungi (the minimum number of sequences required to normalize the differences in sequencing depth) using QIME.

2.6 Multifunctional analysis

The ecosystem multifunctionality index includes three components: soil chemical functions (SCF), soil physical functions (SPF) and plant functions (PF). In the present study, we assessed thirteen ecosystem functions, including SOC, TN, TP, TK, NO₃⁻, NH₄⁺, aP, aK, soil pH, soil water content, soil bulk density, chlorophyll content, and vine pruning weight. N, P and K contents in soil often restrict the primary production of terrestrial ecosystems. NO₃⁻ and NH₄⁺ determinations provide two important mineral nitrogen sources. aP and aK evaluate the direct sources of P and K needed by vines. Chlorophyll is a good indicator of nutritional stress, photosynthetic capacity, and growth status of vines.

Several methods can be used to calculate the multifunctionality index, including single functions, turnover, averaging, and thresholds (including single thresholds and multiple thresholds)[28]. Each method has its own advantages and disadvantages. For example, the average approach is simple and intuitive, while one of the drawbacks of the turnover approach is that some functions may be affected by one or several species. The averaging and threshold approaches have been commonly used[29-31]. To allow
for comparisons with other studies, averaging and multiple-threshold multifunctionality were calculated in this study. The averaging approach is to obtain a single index by standardizing and averaging the different functions \[^{[32]}\]. Z-score transformation was carried out to standardize the data of soil microbial diversity and ecosystem functions \[^{[32]}\]. The multiple-thresholds approach was carried out with a continuous gradient of thresholds, examining the slope of the fitted curve at different thresholds. The “multifunc” package \[^{[26]}\] in R software 3.6 was used to calculate multifunctionality indices.

2.7 Statistical analyses

One-way ANOVAs were conducted to assess the differences in soil properties, quality of grape berries and resulting wine, alpha diversity of soil bacterial and fungal communities, and ecosystem multifunctionality. Duncan’s tests were used for multiple comparisons. Differences were considered significant at \( p < 0.05 \). The Spearman method was used in R software 3.6 to determine the correlations of soil bacterial and fungal diversity with multiple ecosystem functions and the correlations of multiple ecosystem functions with wine quality. A principal coordinate analysis (PCoA) was performed to evaluate the differences in bacterial and fungal community structure among vineyards based on Bray-Curtis distances. A redundancy analysis (RDA) was conducted using R software to analyze the responses of multiple ecosystem functions to the wine quality from each vineyard. A heatmap was drawn with R software 3.6. Graphs were plotted using Origin 9.0.

3 Results

3.1 Soil microbial community composition

3.1.1 Alpha diversity of soil bacterial and fungal communities

The number of bacterial OTUs did not show significant differences among these vineyards (Table 2). The Chaol index for bacterial communities showed a different pattern from that of the variation in bacterial OTUs and was highest at GL vineyard. The Shannon index, showing the diversity of microbial communities, was lower for the bacterial community at HFJR vineyard than the other vineyards. The Simpson index for the bacterial community also showed the lowest value at HFJR vineyard, as did the Shannon index. The number of OTUs, Chaol estimator, and Shannon diversity of the fungal communities varied among the fifteen vineyards. The greatest number of fungal OTUs was at LS vineyard, greater than those at XYWQ, GL, HL, BGS, XG, HD, and HFJR vineyards. The fungal Chaol index showed a pattern that was similar to the trend of fungal OTUs and was affected by latitude, with the highest value at LS vineyard. The Shannon index of the fungal community did not show a significant difference among the vineyards in these main wine sub-regions, including Shizuishan, Yinchuan and Yuquanying.

### Table 2 Alpha diversities of soil bacterial and fungal community of fifteen vineyards

<table>
<thead>
<tr>
<th>Vineyard</th>
<th>Chaol</th>
<th>Shannon</th>
<th>Pioclo</th>
<th>Observed OTUs</th>
<th>Goods_coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYWQ</td>
<td>405.73±20.51(^a)</td>
<td>0.84±0.08(^a)</td>
<td>4.88±0.81(^a)</td>
<td>0.52±0.09(^a)</td>
<td>402±20.78(^a)</td>
</tr>
<tr>
<td>HED</td>
<td>515.73±13.68(^a)</td>
<td>0.88±0.07(^a)</td>
<td>4.58±0.65(^a)</td>
<td>0.54±0.06(^a)</td>
<td>510±13.19(^a)</td>
</tr>
<tr>
<td>GL</td>
<td>390.25±22.34(^a)</td>
<td>0.85±0.10(^a)</td>
<td>4.47±0.82(^a)</td>
<td>0.52±0.09(^a)</td>
<td>382±23.79(^a)</td>
</tr>
<tr>
<td>HL</td>
<td>423.63±56.38(^a)</td>
<td>0.91±0.07(^a)</td>
<td>5.05±0.82(^a)</td>
<td>0.58±0.08(^a)</td>
<td>419±55.31(^a)</td>
</tr>
<tr>
<td>ZY</td>
<td>543.55±39.25(^a)</td>
<td>0.93±0.03(^a)</td>
<td>5.38±0.44(^a)</td>
<td>0.59±0.04(^a)</td>
<td>539±37.36(^a)</td>
</tr>
<tr>
<td>LL</td>
<td>463.81±50.05(^a)</td>
<td>0.88±0.08(^a)</td>
<td>4.99±0.72(^a)</td>
<td>0.57±0.09(^a)</td>
<td>455±142.12(^a)</td>
</tr>
<tr>
<td>XXW</td>
<td>606.47±55.03(^a)</td>
<td>0.85±0.06(^a)</td>
<td>4.87±0.62(^a)</td>
<td>0.53±0.07(^a)</td>
<td>596±51.82(^a)</td>
</tr>
<tr>
<td>BGS</td>
<td>585.82±20.28(^a)</td>
<td>0.94±0.01(^a)</td>
<td>5.81±0.50(^a)</td>
<td>0.64±0.02(^a)</td>
<td>580±198.44(^a)</td>
</tr>
<tr>
<td>YH</td>
<td>413.74±69.61(^a)</td>
<td>0.91±0.04(^a)</td>
<td>5.16±0.68(^a)</td>
<td>0.60±0.06(^a)</td>
<td>409±69.40(^a)</td>
</tr>
<tr>
<td>YM</td>
<td>508.84±46.35(^a)</td>
<td>0.95±0.01(^a)</td>
<td>5.90±0.26(^a)</td>
<td>0.66±0.03(^a)</td>
<td>503±44.94(^a)</td>
</tr>
<tr>
<td>HD</td>
<td>379.70±56.32(^a)</td>
<td>0.78±0.04(^a)</td>
<td>3.69±0.36(^a)</td>
<td>0.43±0.03(^a)</td>
<td>389±56.38(^a)</td>
</tr>
<tr>
<td>HFJR</td>
<td>415.99±79.39(^a)</td>
<td>0.92±0.05(^a)</td>
<td>5.21±0.70(^a)</td>
<td>0.60±0.09(^a)</td>
<td>408±80.61(^a)</td>
</tr>
<tr>
<td>LS</td>
<td>709.17±398.60(^a)</td>
<td>0.95±0.00(^a)</td>
<td>6.19±0.19(^a)</td>
<td>0.67±0.04(^a)</td>
<td>701±388.67(^a)</td>
</tr>
</tbody>
</table>

Note: XYWQ: Xiyiwan; HED: Hedong; GL: Guanlan; HL: Helu; ZY: Zhangyu; LL: Lilan; XXW: Xixiawan; BGS: Baoleijia; BGS: Bagesi; YH: Yuhuang; YM: Yuma; XG: Xige; HD: Huida; HFJR: Hongfenjiarong; LS: Luoshan. Different lowercase letters indicate significant differences according to Duncan’s multiple range test at the \( p < 0.05 \) level. Results are reported as the mean ± SD (\( n = 3 \)).

3.1.2 Soil bacterial and fungal community composition

*Actinobacteria* was the most abundant bacterial phylum among these sites, accounting for 32.38% of all sequences on average, followed by *Proteobacteria* (30.62%), *Chloroflexi* (11.66%),...
Acidobacteria (8.12%), and Gammaproteobacteria (5.81%) (Figure 2a). The relative abundance of Actinobacteria was highest at XYWQ and GL vineyards. However, the relative abundance of Proteobacteria in vineyards from Qingtongxia and Hongsipu regions was higher than that at vineyards from Shizuishan and Yinchuan regions. PCoA based on Bray-Curtis distances was applied to visualize the overall patterns of bacterial and fungal community composition at each vineyard from these five wine sub-regions (Figure 3). For bacterial communities (Figure 3a), all vineyards tended to be separated, which indicated that bacterial community structure has differentiated among the vineyards.

Ascomycota was the dominant fungal phylum (82.98%), followed by Mortierellomycota (7.72%) and Basidiomycota (7.06%) (Figure 2b). The abundances of these three phyla significantly differed (p<0.05) among vineyards from different locations. According to PCoA (Figure 3b), the fungal communities of some vineyards, such as YH and BLLJ, tended to be grouped together, which indicated that they shared similar bacterial community structure. Overall, the fungal community composition at different sites was not totally affected by vineyard location, indicating that fungal community composition was less affected than bacterial community composition.


Figure 2  Relative abundance of soil bacterial (a) and fungal (b) community based on phylum level of grape vineyards


Figure 3  Principal coordinates analysis (PCoA) of microbial community composition based on soil bacterial (a) and fungal (b) relative abundance of OTUs
3.2 Ecosystem multifunctionality and its relationship with microbial diversity

The ecosystem multifunctionality index calculated by the averaging method was highest at LS vineyard and lowest in HL vineyard (Figure 4a). Values at LL, XXW, BLLJ, and BGS vineyards from the same region were all positive. Overall, ecosystem multifunctionality did not show obvious differences among the vineyards. Soil chemical functions were also similar among the vineyards (Figure 5). Soil physical functions showed a similar pattern at vineyards from Shizuishan, Yinchuan, and Yuquanying regions but an opposite pattern at vineyards from Qingtongxia and Hongshiku regions (Figure 6).

Figure 4  Average multifunctionality index of fifteen vineyards along the latitude gradients (a) and the relationship between average multifunctionality indices with microbial diversity (b)

Correlation analysis revealed that the bacterial diversity (BD) was not significantly correlated with average multifunctionality (AMF) but was significantly correlated with SPF (Figure 4b). Fungal diversity (FD) was significantly positively correlated with the AMF of ecosystems and SCF and negatively correlated with SPF.

According to the multiple-threshold method (Figure 7), $T_{\text{min}}$, the minimum threshold when diversity influences multifunctionality, was 23% for the bacterial community and 9% for the fungal community. $T_{\text{max}}$, the maximum threshold when diversity has no effect on multifunctionality, was 41% for the bacterial community and 34% for the fungal community. Under the condition of a threshold below 75%, the slope of ecosystem multifunctionality and diversity was positive for the bacterial community, indicating that ecosystem multifunctionality was maintained by bacterial diversity. Under the condition of a threshold exceeding 75%, the slope of the relationship between ecosystem multifunctionality and fungal community diversity was positive, revealing that ecosystem multifunctionality was maintained by fungal diversity.

3.3 Quality of grape berries and the resulting wine

3.3.1 Quality of grape berries

From the results of grape quality analysis (Table 3), the total sugar content of grape berries was close to or greater than 200 g/L, indicating that the grapes of each vineyard ripened well in this study. The total sugar content of grapes was higher at YM vineyard than at the other sites, while the titratable acidity content was higher at YH, HD, and LS vineyards than at the other vineyards. The pH of grape berries showed a pattern different from those for total sugar and titratable acidity, with the highest value at BGS vineyard.

Figure 5  Average multifunctionality index of soil chemical properties of fifteen vineyards along the latitude gradients

Figure 6  Average multifunctionality index of soil physical properties of fifteen vineyards along the latitude gradients


Correlation analysis revealed that the bacterial diversity (BD) was not significantly correlated with average multifunctionality (AMF) but was significantly correlated with SPF (Figure 4b). Fungal diversity (FD) was significantly positively correlated with the AMF of ecosystems and SCF and negatively correlated with SPF. According to the multiple-threshold method (Figure 7), $T_{\text{min}}$, the minimum threshold when diversity influences multifunctionality, was 23% for the bacterial community and 9% for the fungal community. $T_{\text{max}}$, the maximum threshold when diversity has no effect on multifunctionality, was 41% for the bacterial community and 34% for the fungal community. Under the condition of a threshold below 75%, the slope of ecosystem multifunctionality and diversity was positive for the bacterial community, indicating that ecosystem multifunctionality was maintained by bacterial diversity. Under the condition of a threshold exceeding 75%, the slope of the relationship between ecosystem multifunctionality and fungal community diversity was positive, revealing that ecosystem multifunctionality was maintained by fungal diversity.

3.3.2 Wine quality

As presented in Table 4, the alcohol content of wine ranged from 12.86% to 16.03% (v/v), with the highest value at YM vineyard. Vineyards from the Yinchuan region exhibited the highest values for total extract, residual sugar, total tannins, total

flavonoids, total phenolics and titratable acidity of wine. Moreover, the total tannin content, total anthocyanin content, total phenolics content, and titratable acidity were all highest at GL vineyard, with 717.64 mg/L, 4688.51 mg/L, 702 mg/L, and 7.53 g/L, respectively. Interestingly, the lowest values for total extract, residual sugar, total flavonoids, and phenolics content were all at sites in the Hongsipu region. The residual sugar content ranged between 0.93 g/L at HD vineyard and 2.43 g/L at HL vineyard. The total flavan-3-ol content ranged between 290.17 mg/L at LL vineyard and 944.45 mg/L at HD vineyard. The titratable acidity ranged from 5.23 g/L at HL vineyard to 7.53 g/L at GL vineyard.

![Image](https://www.ijabe.org)

**Figure 7** Relationships between diversity of bacteria (a, b) and fungi (c, d) and the number of functions beyond a threshold of maximum observed value

<table>
<thead>
<tr>
<th>Vineyard</th>
<th>Titratable acidity/g L⁻¹</th>
<th>Total sugar/g L⁻¹</th>
<th>pH</th>
<th>Vineyard</th>
<th>Titratable acidity/g L⁻¹</th>
<th>Total sugar/g L⁻¹</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYWQ</td>
<td>4.48±0.04³</td>
<td>242.17±5.97b</td>
<td>3.95±0.01³</td>
<td>HGS</td>
<td>4.09±0.11²</td>
<td>226.33±15.12b</td>
<td>4.10±0.02³</td>
</tr>
<tr>
<td>HED</td>
<td>4.76±0.04³</td>
<td>207.33±17.56©</td>
<td>3.77±0.00²</td>
<td>YM</td>
<td>4.28±0.11²</td>
<td>258.66±6.01²</td>
<td>4.03±0.02³</td>
</tr>
<tr>
<td>GL</td>
<td>4.73±0.03³</td>
<td>219.17±4.51e</td>
<td>3.72±0.01³</td>
<td>GL</td>
<td>4.21±0.11²</td>
<td>230.83±11.84*</td>
<td>3.97±0.04³</td>
</tr>
<tr>
<td>HL</td>
<td>5.00±0.06³</td>
<td>197.00±9.73f</td>
<td>3.92±0.01³</td>
<td>ZY</td>
<td>4.21±0.11²</td>
<td>215.67±2.47e</td>
<td>3.59±0.00³</td>
</tr>
<tr>
<td>LL</td>
<td>4.24±0.09³</td>
<td>205.00±10.33d</td>
<td>3.72±0.01³</td>
<td>LL</td>
<td>4.87±0.23³</td>
<td>227.33±12.58²</td>
<td>3.62±0.00³</td>
</tr>
<tr>
<td>XXW</td>
<td>4.42±0.14³</td>
<td>223.67±2.08c</td>
<td>3.70±0.02³</td>
<td>XXW</td>
<td>4.48±0.04³</td>
<td>242.17±5.97b</td>
<td>3.95±0.01³</td>
</tr>
<tr>
<td>BLLJ</td>
<td>4.18±0.05³</td>
<td>226.67±1.76c</td>
<td>3.70±0.02³</td>
<td>BLLJ</td>
<td>4.48±0.04³</td>
<td>242.17±5.97b</td>
<td>3.95±0.01³</td>
</tr>
</tbody>
</table>

Note: XYWQ: Xiuyangwanqian; HED: Hedong; GL: Guilan; HL: Helu; ZY: Zhangyu; LL: Lilan; XXW: Xixiawanqian; BLLJ: Baolelijia; HGS: Bagesi; YH: Yuhuang; YM: Yuma; XXW: Xige; HD: Huida; HFJR: Hongfenjiarong; LS: Luoshan. Different lowercase letters indicate significant differences according to Duncan’s multiple range test at the p < 0.05 level. Results are reported as the mean ± SD (n = 3).

<table>
<thead>
<tr>
<th>Vineyard</th>
<th>Alcoholity %, v/v</th>
<th>Total extract /g L⁻¹</th>
<th>RS/g L⁻¹</th>
<th>Total flavan-3-ol /g L⁻¹</th>
<th>Total tannin /mg L⁻¹</th>
<th>Total anthocyanins /mg L⁻¹</th>
<th>Total flavonoids /mg L⁻¹</th>
<th>Total phenolics /mg L⁻¹</th>
<th>TA/g L⁻¹</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>XYWQ</td>
<td>13.33±0.05³</td>
<td>32.47±0.42c</td>
<td>1.70±0.03³</td>
<td>709.58±22.35</td>
<td>358.31±21.71</td>
<td>181.45±15.34</td>
<td>319.17±14.72</td>
<td>528.85±37.72</td>
<td>6.18±0.07³</td>
<td>3.46±0.01³</td>
</tr>
<tr>
<td>HED</td>
<td>14.34±0.18c</td>
<td>32.50±0.17</td>
<td>1.78±0.01³</td>
<td>642.21±24.96</td>
<td>313.54±33.71</td>
<td>424.39±12.20</td>
<td>289.44±11.40</td>
<td>464.60±37.68</td>
<td>5.29±0.06³</td>
<td>3.72±0.02³</td>
</tr>
<tr>
<td>GL</td>
<td>14.18±0.06c</td>
<td>38.00±0.00f</td>
<td>1.59±0.04³</td>
<td>850.66±12.04</td>
<td>717.64±13.89</td>
<td>148.34±18.45</td>
<td>468.51±18.55</td>
<td>702.98±35.80</td>
<td>7.53±0.01³</td>
<td>3.20±0.02³</td>
</tr>
<tr>
<td>HL</td>
<td>14.00±0.03³</td>
<td>37.90±0.33d</td>
<td>2.43±0.05³</td>
<td>854.83±18.66</td>
<td>621.82±49.99</td>
<td>240.13±5.05</td>
<td>453.19±9.34</td>
<td>664.47±15.12</td>
<td>5.23±0.09³</td>
<td>3.79±0.01³</td>
</tr>
<tr>
<td>ZY</td>
<td>12.86±0.05³</td>
<td>32.10±0.17f</td>
<td>1.00±0.02³</td>
<td>722.11±36.41</td>
<td>429.39±23.54</td>
<td>225.21±26.53</td>
<td>338.29±56.77</td>
<td>533.58±9.74</td>
<td>5.65±0.06³</td>
<td>3.71±0.44³</td>
</tr>
<tr>
<td>LL</td>
<td>13.95±0.05³</td>
<td>31.27±0.25f</td>
<td>1.07±0.04³</td>
<td>290.17±34.50</td>
<td>457.13±13.15</td>
<td>235.37±13.21</td>
<td>259.39±21.22</td>
<td>434.47±5.99</td>
<td>5.97±0.00³</td>
<td>3.37±0.01³</td>
</tr>
<tr>
<td>XXW</td>
<td>12.97±0.05³</td>
<td>29.90±0.17f</td>
<td>1.17±0.03³</td>
<td>563.96±77.44</td>
<td>427.78±32.43</td>
<td>234.84±11.86</td>
<td>355.64±82.28</td>
<td>566.84±22.84</td>
<td>5.71±0.09³</td>
<td>3.54±0.01³</td>
</tr>
</tbody>
</table>
ntho represents total
tightly affected wine quality
t acquisition, such as copiotrophic
AMF and SCF showed significant
wine quality and aspects of ecosystem mult
AMF, SCF, PF, and SPF (\textit{Note:}
AMF: average multifunctionality; SCF: soil
chemical multifunctionality; SPF: soil
physical multifunctionality; PF: plant
multifunctionality.

Note: Alcohol represents alcoholicty; Acidity represents titratable acidity;
Extract represents total extract content; Flavan represents total flavan-3-ols
content; Tannin represents total tannin content; Antho represents total
anthocyanin content; Flavonoids represents total flavonoids content; Phenolics
represents total phenolic content; AMF: average multifunctionality; SCF: soil
chemical multifunctionality; SPF: soil physical multifunctionality; PF: plant
multifunctionality. * indicated significant correlations (p<0.05).

Figure 8 Heatmap of correlation between wine quality and ecosystem multifunctionality (Z score)

The RDA ordination plot showed the relationships between
wine quality and aspects of ecosystem multifunctionality, including
AMF, SCF, PF, and SPF (Figure 9). The first and second axes explained 11.08\% and 4.76\% of the variation, respectively. Overall, these ecosystem functions explained wine quality (p<0.05). These multifunctionalities all significantly affected wine quality (p<0.05). Similar to the results of correlation analysis, AMF and SCF exhibited significant positive correlations with the acidity of wine.

3.4 Relationships between wine quality and multifunctionality

As shown in Figure 8, AMF and SCF showed significant positive correlations with acidity of wine, while AMF and SCF displayed significant negative correlations with wine pH. AMF, PF, and SCF all had significant negative correlations with residual sugar content. The results of the multiple threshold approach are shown in Figure 7. In addition, PF was significantly negatively correlated with extract content. SPF exhibited significant negative correlations with alcohol and total anthocyanin content of the resulting wine.

Note: XYWQ: Xiyuwangquan; HED: Hedong; GL: Guanlan; HL: Helu; ZY: Zhangyu; LL: Lilan; XXW: Xixiawang; BLLJ: Baolelijia; BGS: Bagesi; YH: Yuhuang; YM: Yuma; XG: Xige; HD: Huida; HFJR: Hongfenjiarong; LS: Luoshan. RS: Residual sugar. TA: Titratable acidity. Different lowercase letters indicate significant differences according to Duncan’s multiple range test at the p<0.05 level. Results are reported as the mean ± SD (n=3).

4 Discussion

4.1 Soil microbial community structure of vineyard

The profiles of both bacterial and fungal communities showed significant differences among vineyards (Figures 2, 3, and Table 2). The bacterial community structure was different among the fifteen vineyards, while the differences in the fungal community structure were not as obvious (Figure 2). In addition, the Shannon diversity of the bacterial and fungal communities showed different patterns. The lowest bacterial diversity was at HFJR vineyard, and there was no significant difference in the Shannon diversity of the bacterial community among the other vineyards. However, fungal Shannon diversity varied more significantly. These results were consistent with findings from previous work conducted at vineyards in Xinjiang showing that the Shannon diversity of fungal communities showed greater variation than that of bacterial communities\cite{133}. Microbial community structure is affected by soil, climate, plant type and diversity, and other factors. The ecoregographical conditions for suitable wine grape-planting areas are complex and diverse. Moreover, \textit{Actinobacteria} and \textit{Proteobacteria} were the dominant bacterial phyla in each vineyard, which was consistent with Wei et al.\cite{134}. Microbial taxa with different strategies for nutrient acquisition, such as copiotrophic and oligotrophic groups, are impacted by soil conditions.
oligotrophic hypothesis states that nutrient-rich conditions are beneficial for copiotrophic but not oligotrophic bacterial growth[31]. In the present study, the relative abundance of Acidobacteria, considered an oligotrophic group, was much higher than the relative abundance of Firmicutes, which is classified as a copiotrophic group. This is consistent with the poor quality of soil in northwest China. Ascomycota was the main fungal phylum in each vineyard, followed by Basidiomycota and Mortierellomycota. Basidiomycetes are typically saprotrophic and very sensitive to organic matter decomposition, especially plant litter with high lignin content[36,37]. There was very little plant litter in the human-modified ecosystems under study, leading to a relatively lower abundance of Basidiomycota. The soil microbial community composition and structure were affected by climate, soil fertility and other factors in different wine grape-producing areas of Xinjiang, which was supported by the results of several previous studies[38,39].

4.2 Drivers of ecosystem multifunctionality

The changing patterns of ecosystem multifunctionality and soil functions of each vineyard and their relationships with bacterial and fungal diversity were explored. In the current study, as these approaches have their own advantages and disadvantages[23], two metrics, averaging and multiple thresholds, were selected to calculate multifunctionality indices. With the method of averaging, the multiple ecosystem functions differed significantly among the experimental vineyards, illustrating an imbalance in the tradeoffs among single functions of these vineyards[40]. This was because vineyards from different locations varied in soil biological traits, soil physical traits, and soil nutrient contents, resulting in differences in grape properties. More importantly, only the vineyards from Yuquanying exhibited overall positive values, and the ecosystem multifunctionality of vineyards from different wine sub-regions showed different patterns, which reflects that the ecosystem multifunctionality of vineyards may depend more on specific environmental factors at a fine scale, especially the microclimatic and soil conditions in the vineyard.

Some previous studies have illustrated that microbial diversity promotes ecosystem multifunctionality in the natural environment[41,43], which also indicated that variations in bacterial and fungal diversity could further influence multifunctionality. Ecosystems with single plant types, such as farming systems or economic forest systems, have lower biodiversity than less disturbed or natural terrestrial ecosystems[42]. Jing et al.[10] found that the organic fertilizer application increased soil multifunctionality by positively promoting both bacterial and fungal diversity. In this study, fungal diversity rather than bacterial diversity showed a positive relationship with ecosystem multifunctionality in the vineyard. Consequently, any alternations in fungal diversity resulting from field management practices[11,43,44], such as pesticide application, fertilization, and residue management, may affect multifunctionality by shifting fungal diversity. Furthermore, positive correlations between soil bacterial diversity and some single functions, such as SPF, were found (Figure 4). However, bacterial diversity could not maintain ecosystem multifunctionality. These results indicate that the effect of bacterial diversity may not be strong enough to influence overall multifunctionality in the experimental vineyards. Fungal diversity showed a significant positive relationship with SCF and a negative relationship with SPF.

4.3 Response of wine quality to ecosystem multifunctionality

In the current study, ecosystem multifunctionality was found to have different relationships with various indices of wine quality, such as being positively correlated with titratable acidity and negatively correlated with pH and residual sugar content of wine. In addition, indices of wine quality showed different responses to different functions. For example, acidity responded positively, and pH and residual sugar content of wine responded negatively to SCF, while both the alcohol and total anthocyanin content of wine responded negatively to SPF. This was because the soil water content included in SPF was negatively correlated with the total anthocyanin content of wine[45]. It was reported that wine acidity was positively affected by soil pH and soil water content but negatively affected by soil organic matter content. In this study, wine acidity was positively correlated with SCF, including soil organic carbon content and pH, showing the tradeoffs among these factors affecting acidity. Furthermore, the phenolics content of wine has been reported to be affected by the soil N content and positively affected by the K content[46], thus, there was no significant relationship between multifunctionality and the phenolics content in this study.

5 Conclusions

This study shows that microbial community structure, ecosystem multifunctionality, and wine quality varied among the experimental vineyards, showing different trends in different wine sub-regions affected by different specific factors at a fine scale. Moreover, our findings provide experimental evidence that is consistent with previous results showing that fungal diversity promotes the multifunctionality of vineyards. However, bacterial diversity was not able to explain ecosystem multifunctionality as was fungal diversity. Moreover, different multifunctionality indices promote different aspects of wine quality, showing the effects of tradeoffs among functions on wine quality. The results reveal relevant ecological relationships between biodiversity and ecosystem functions in vineyards. In addition, it is suggested that management practices that are beneficial for fungal diversity should be selected to maintain ecosystem functions in vineyards.

Acknowledgements

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References

Fungal diversity drives the ecosystem multifunctionality of vineyards in a semi-arid region


