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# New insights into the impact of wood vinegar on the growth and rhizosphere microorganisms of Cherry Radish (*Raphanus Sativus*)

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Understanding the impact of wood vinegar on the growth of cherry radish is indispensable for its use in crop cultivation and evaluating its biosafety. Our study explored the regulation of rhizosphere microbial abundance and activity by wood vinegar, as well as the relationship between microbial community and growth factors in-depth and systematically. Bacterial communities at the phylum and genus levels were significantly changed after wood vinegar treatment. The abundance of *Actinobacteriota* and *Firmicutes* increased, while Proteobacteria was promoted in high carbon soil by wood vinegar application. Fungi positively responded to radish root traits and were correlated with aboveground biomass and fruit production. The fungi that correlated with photosynthesis included Albifimbria, Allomyces, Calcarisporiella, Clonostachys, Fusarium, Fusicolla, Knufia, Nigrospora, Paraconiothyrium, Preussia, Talaromyces, and Mortierellomycota. Wood vinegar treatment significantly affected the composition and abundance of soil bacterial and fungal communities in radish rhizosphere. The promotion of radish growth by wood vinegar may be attributed to the stimulation of soil microorganisms that degraded aromatic compounds and drove nitrogen cycling. This study provided novel insights into the significant promotion of radish growth by wood vinegar and identified potential microbial targets for agricultural applications.

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#### 16 ABSTRACT

17	Understanding the impact of wood vinegar on the growth of cherry radish is indispensable
18	for its use in crop cultivation and evaluating its biosafety. Our study explored the regulation of
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21	the phylum and genus levels were significantly changed after wood vinegar treatment. The
22	abundance of Actinobacteriota and Firmicutes increased, while Proteobacteria was promoted in
23	high carbon soil by wood vinegar application. Fungi positively responded to radish root traits and
24	were correlated with aboveground biomass and fruit production. The fungi that correlated with
25	photosynthesis included Albifimbria, Allomyces, Calcarisporiella, Clonostachys, Fusarium,
26	Fusicolla, Knufia, Nigrospora, Paraconiothyrium, Preussia, Talaromyces, and
27	Mortierellomycota. Wood vinegar treatment significantly affected the composition and abundance
28	of soil bacterial and fungal communities in radish rhizosphere. The promotion of radish growth by
29	wood vinegar may be attributed to the stimulation of soil microorganisms that degraded aromatic
30	compounds and drove nitrogen cycling. This study provided novel insights into the significant
31	promotion of radish growth by wood vinegar and identified potential microbial targets for
32	agricultural applications.

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36 **Key words:** Wood vinegar; cherry radish; yield; photosynthesis; microorganism.



#### 1. Introduction

Agricultural and forestry waste, which is approximately 2 billion tons generated annually in 38 39 China (Fan et al. 2020), is an important biomass resource as a byproduct generated during the production and processing of agroforestry (Ozturk et al. 2017). Wood vinegar (WV) obtained via 40 condensing and separating the flue gas generated from biomass pyrolysis (Hagner et al. 2013), 41 42 which has a wide range of applications in agriculture, environmental protection, and healthcare (Mahmud et al. 2018; Grewal et al. 2018; Hu and Gholizadeh, 2020; Sarchami et al. 2021). 43 Mmojieje et al. (2015) reported that WV from mixed wood biomass exhibited up to 90% mortality 44 for red spider mite and green peach aphid. Gao et al. (2020) reported that wheat straw vinegar 45 containing phenols, acetic acid, and cations significantly decreased the wheat fusarium head blight 46 infection rate and deoxynivalenol content by 66% and 69%, respectively. Recently, research on 47 the application of WVs has mainly focused on their antibacterial properties and insecticidal effects 48 in agriculture. Nevertheless, the sawdust vinegar from low temperature (< 400 °C) can be used as 49 liquid fertilizer to promote wheat seeds germination (Shang et al. 2021). These results almost 50 focused on the biological response of WV to the target substance (plants, pests, pathogens), but 51 52 the rhizosphere microbial behavior of WV was largely overlooked. WV, as soil remediation agents, consisted of alcohols, esters, amines, pyridines, as well as 53 trace elements such as K, P, Ca, Mn and Fe (Hou et al. 2018; Lu et al. 2020). As reported by many 54 previous studies, WV could increase soil fertility by increasing nutrient elements (e.g., nitrogen 55 (N), phosphorus (P), and potassium (K)) (Polthanee et al. 2015; Sun et al. 2018), reducing NH<sub>3</sub> 56 volatilization (Win et al. 2009), and dissolved organic molecules (Lashari et al. 2013; Lashari et 57



al. 2015), resulting in improved production of crop. WV gotten from biomass pyrolysis at low 58 temperature (< 150 °C) are mainly composed of acid compounds, which promote the length of 59 wheat main roots to nearly 1.2 times (Lu et al. 2019). Lashari et al. (2013) reported that biochar 60 poultry manure compost-WV amendment significantly increased wheat yield through the effect of 61 WV on leaching soluble salts and enhancing the effectiveness of P and K. Moreover, the dose of 62 3.0% mL hazelnut shell WV had positive effects on the number of bacteria and β-glucosidase 63 enzyme activity in soil (Koc et al. 2019). To our best knowledge, most of the previous study 64 focused on the effect of WV on soil microorganisms and plant growth singly (Jeong et al. 2015), 65 and no reports was conducted to evaluate the regulation of rhizosphere microorganisms through 66 WV leading to crop growth. Therefore, the effects of WV composition on the response of soil 67 microorganisms and the promotion of cherry radish (Raphanus Sativus) growth were studied, and 68 69 its applications were further determined. In this study, rice straw as the raw material and were successfully extracted WV through 70 pyrolysis. We deeply explored the influence of WV on the rhizosphere microbial community of 71 cherry radish through soil culture experiments. In addition, the effects WV concentrations on the 72 growth indicators, photosynthetic efficiency, and chlorophyll content of radish were also explored. 73 Exploring whether WV has the effect of activating rhizosphere microorganisms, and thus 74 stimulating the absorption of nutrients by crops in the rhizosphere. These results could provide 75 new insights into the resource utilization of agricultural waste, and important support for green 76 agricultural development. 77



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#### 2. Materials and methods

#### 79 **2.1 Materials**

The cultivated brown soil has a pH value of 6.5, 32.38 g/kg of organic matter, 15.12 g/kg of total nitrogen, 15.26 mg/kg of available phosphorus and 56.58 mg/kg available potassium. The

"Red Angel" cherry radish used as the experiment crop and was purchased from Beijing

Dongsheng Seed Industry Co., Ltd. When rice straw is heated to a temperature of 450°C, a

condensed liquid known as wood vinegar is collected. Once standing, the clarified wood vinegar

in the middle is taken.

#### 2.2 Experimental design

Cherry radish seeds in a 5% (v/v) NaClO for 10 minutes for disinfection, and rinsed with 87 clean water several times to remove residual disinfectants (Gu et al. 2021). Firstly, five seeded 88 seeds were sown directly at a depth of 1 cm on the surface of bowl soil (3 kg). After 7 days of 89 seedling growth, excess plants were removed, leaving one plant with good and consistent growth 90 in each pot. After 45 days of growth, the cherry radish was harvested and related indicators were measured in the soil. Each group of treatments was set with 5 replicates, and a total of 5 treatments were set in the experiment: the untreated wood vinegar was set as the control (CK), and the wood vinegar was diluted 400 (W400), 300 (W300), 200 (W200), 100 (W100), and 50 (W50) times, 94 95 respectively. During the experiment, 500 mL of wood vinegar with different dilution ratios were 96 poured into pots and bowls, and the same volume of water was poured into CK.

#### 2.3 Characterization of Soil and WV

The soil pH is measured using the potential method (soil-water ratio 2.5:1). The organic



matter of the soil was determined using potassium dichromate-sulfuric acid (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub>) for oxidation and ferrous sulfate (FeSO<sub>4</sub>) titration (Peng et al. 2016). The soil sample is digested using copper sulfate (CuSO<sub>4</sub>) and potassium sulfate (K<sub>2</sub>SO<sub>4</sub>), and then titrated with hydrochloric acid (HCl) to obtain total nitrogen (Lenaerts et al. 2018). The available phosphorus and potassium were determined by modified kelowna methods (Qian et al. 1994).

The molecular composition of WV samples measured using an electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) (Bruker SolariX, Bruker, Germany) equipped with a 9.4 T superconducting magnet. Sample solutions were injected into the electrospray source at 180  $\mu$ L/h with a syringe. The operating conditions for negative-ion formation be applied with a 4.0 kV spray shield voltage, 4.5 kV capillary column introduced voltage, and 320 V capillary column end voltage. The mass range was configured with 200–800 Mass to charge ratio (m/z). The samples were performed 128 FT-ICR MS scans to improve signal-to-noise ratio and dynamic range. The detailed determinations of H/C, O/C, and double bond equivalents (DBE) of molecular compositions are presented in Text S1 (Hua et al. 2019).

#### 2.4 Measurements of Plant Growth

Cherry radish fruit was harvested after ripening, and the diameter of the fruit is measured using a ruler (centimeters) and the yield is calculated. After harvest, the root morphology of cherry radish treated with different doses of WV was analyzed using the WinRhizo system v.4.0b (Instruments Regent LA2400, Japan). The photosynthetic index was measured on a sunny morning (9:00-11:00), and the second leaf at the top of the plant was selected as the measured leaf. (Gu et al., 2021) The net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and



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intracellular CO<sub>2</sub> concentrations (Ci) of cherry radish were analyzed by the CIRAS-3 portable gas exchange system (PP-Systems, USA) (Liu et al. 2020). The supernatant was separated and the absorbance was recorded at 662 nm for Chlorophyll a and 646 nm for Chlorophyll b using the Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) (Şükran et al. 1998).

#### 2.5 Microbial diversity and community

The DNA of soil bacteria was extracted from 0.5 g of frozen soil using the FastDNA SPIN Kit (MP Biomedicals, CA, USA). The 16S rRNA gene was amplified through thermocycler polymerase chain reaction (PCR) system (GeneAmp 9700, ABI, USA) using 16S rRNA amplicon (338F, 5'-ACTCCTACGGGAGGCAGCAG-3')/806R, 5'-GGAC TACHVGGGTWTCTAAT-3'). Then, the quality and concentration of the extracted DNA were determined using a NanoDrop ND-1000 UV-Vis spectrophotometer (Thermo Fisher, Waltham, MA, USA) and a Quanti Fluor dsDNA system (Promega, Madison, WI, USA). Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) used the Illumina MiSeq platform (Illumina, San Diego, USA) to analyze the bacterial diversity according to the standard protocols. The online Majorbio Cloud Platform (http://www.majorbio.com) was used to perform data analysis, which included Venn diagram analysis, collinearity analysis, alpha diversity analysis, beta diversity analysis, and heatmap analysis.

#### 2.6 Statistical Analysis

One-way ANOVA, correlation analysis, and Duncan's multiple range-test were obtained from 138 SPSS version 25.0 (IBM, Armonk, NY, USA). All the experiments were carried out in triplicate and a significant difference was considered at p < 0.05. Unless otherwise indicated, all other 140



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experiments were conducted in triplicate, and significant differences between the experiments

#### 142 were taken into account (p < 0.05).

#### 3. Results and discussions

#### 3.1 Composition analysis of wood vinegar

The molecular composition of WV from biomass pyrolysis through FT-ICR MS analysis are 145 depicted in Fig. 1. Compared to CHON, CHONS, and CHOS chemicals, CHO compounds contain 146 higher carbon number, oxygen number, and double bond equivalent (DBE value) due to high-147 temperature pyrolysis (Shang et al. 2021). The high number of carbon, oxygen, and DBE of WV 148 were mainly attributed to biomass deoxygenation and bond breaking reaction (Mettler et al. 2012). 149 All detected substances are divided into seven groups, which were shown as follows: (Lian et al. 150 2023) (lipid (H/C: 1.5–2.0; O/C: 0–0.3), protein (H/C: 1.5–2.2; O/C: 0.3–0.67), carbohydrate (H/C: 151 152 1.5–2.2; O/C: 0.67–1.2), unsaturated hydrocarbon (H/C: 0.7–1.5; O/C: 0–0.1), lignin (H/C: 0.7– 1.5; O/C: 0.1–0.67), tannin (H/C: 0–1.5; O/C: 0. 67–1.2), and condensed aromatic-like components 153 (H/C: 0.2–0.7; O/C: 0–0.67)), and the results shown in the Van Krevelen diagram clearly 154 155 illustrated the differences in molecular composition of WV. The main distribution of WV compounds H/C= 0.2-0.7 and H/C= 0.5-1.5, indicating rich aromatic structure and lignin, with 156 45.51% and 22.70% contents, respectively (Fig. 2c-2d). Lignin decomposed at high temperatures 157 to form more lignin/CRAM like structures and aromatic WVs (Qu et al. 2011), which was also 158 supported by previous studies (Qu et al. 2011; Shang et al. 2021). 159

#### 3.2 Wood vinegar promotes the yield of *cherry radish*

161 The effects of WV at different concentrations on the yield indicators of cherry radish were different



and were shown in Fig. 2. Within a certain concentration range (W50-W200), the yield of cherry 162 radish gradually increased with the increased concentration of wood vinegar. When the added 163 concentration of WV reached W200, the diameter and yield of radish reached the largest values, 164 increasing by 42.43% and 44.91% respectively. The application of WV increased soil organic 165 matter, enhanced nutrient uptake by plants, and stimulated populations of beneficial 166 167 microorganisms (Cardelli et al. 2020; Wang et al. 2022). Soil treatment with a 500-fold diluted WV resulted in a significant (P<0.05) 73.39% increase in bacterial population (e.g., Gram-negative 168 bacteria, anaerobic bacteria, and aerobic bacteria) and enhanced soil ecological activity (Rui et al. 169 2014). Simultaneously, WV could also promote the shoot biomass of radish (Fig. S1). After treated 170 by 200-fold diluted WV (W200), the growth rate and biomass accumulation of radish increased 171 by 34.08% compared with CK, which may be related to the regulation of WV on soil microbial 172 activity, and possible by promoting radish nutrient absorption. Overall, the use of WV can improve 173 the growing environment of radish, thereby promoting aboveground growth and development of 174 radish (Mhamdi et al. 2023). 175

#### 3.3. Effect of wood vinegar on Photosynthesis of cherry radish

The effect of WV treatment on the photosynthesis of cherry radish are shown in Fig. 3.

Compared with CK, there was a significant difference in the net photosynthetic rate of cherry radish treated with different concentrations of WV (p<0.05). After applying 200 times WV, the maximum net photosynthetic rate reached 25.53  $\mu$ m CO<sub>2</sub>/(m<sup>2</sup>·s), which was 80.45% and 19.97-101.84% higher than the CK and other WV treatment groups, respectively. The net photosynthesis also verified that the W200 treatment group had the strongest ability to accumulate fruit and



biomass in cherry radish (Fig. 2). WV enhanced net photosynthesis by regulating soil microbial 183 activity and enhancing the ability of crop roots to absorb nutrients (Chen et al. 2016; Rui et al. 184 2014). Similarly, 200 times WV treatment had a positive impact on the transpiration rate of 185 cherries (up to 15.88 mmol H<sub>2</sub>O/(m<sup>2</sup>·s)), accelerating water absorption and transportation (Fig. 186 3b). The transpiration rate of cherries was related with root growth (Kulkarni et al. 2017), and the 187 changed trend of root index in W200 treated cherries further confirmed the enhanced transpiration 188 rates (Fig. S2). For example, after W200 treatment, the contents of root tips, length, and surface 189 area were 35.50, 16.70 cm, and 36.33 cm<sup>2</sup>, respectively, with an increase of 59.19%, 74.14%, and 190 26.72% compared to CK. The stomatal conductance fluctuations were significant, with a 191 significant level (p<0.05) observed in the WV treatment compared to the control, particularly with 192 W200 showing a 92.57% increase (Fig. 3c). As the concentration of WV increased, the stomatal 193 conductance of cherry radishes firstly decreased and then increased, which was consistent with the 194 changes in net photosynthetic rates and transpiration rates. Except for the W50 treatment, the 195 intercellular CO<sub>2</sub> concentration in cherry radish leaves of all other treatment groups was lower 196 197 than that of the CK group, with a decrease range of 13.83%-28.56% (Fig. 3d). The lower the intercellular CO<sub>2</sub> concentration, the more CO<sub>2</sub> was utilized by the leaves for photosynthesis (Gu 198 et al. 2021). The trend of intercellular CO<sub>2</sub> content in different treatment groups were basically the 199 200 opposite of net photosynthetic rate, which was consistent with the laws of plant photosynthesis. In addition, the trends in cherry leaf chlorophyll content and chlorophyll a/b ratio (Fig. S3) were in 201 202 line with the patterns of changed net photosynthetic rates.

3.4 Effects of WV on the Abundance and Diversity of Bacteria and Fungi

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The coverage of the WV treatment group (W200 and W400) and the CK were all above 0.99, indicating that the sequencing results well reflected the actual situation of the bacteria and fungi of the samples (Fig. 4a-4b). The Shannon index of the treatment group with 200-fold diluted WV showed the highest value, indicating that the treatment with 200-fold diluted WV was beneficial to the increase of rhizosphere bacteria diversity, as shown in Fig. 4a-4b. Simpson's index also showed that the bacterial community of the treatment group with 200-fold diluted WV had the lowest Simpson index, which further supports the results of the Shannon index. The results showed that W200 had no significant difference in fungal diversity index (p>0.05), but had extremely significant differences in bacterial Shannon (p < 0.001), Simpson (p < 0.001), and OUT number (p<0.001), indicating that W200 can promote bacterial diversity and richness. Venn diagram could be used to represent the similarity and overlap of soil microorganisms (Fig. S4). The operational taxonomic unit (OTU) number of bacteria and fungi in the soil samples after treated differently were 2047 and 290, respectively. The independent bacteria and fungi OTU numbers in the 200fold diluted (W200) WV treatment were 148 and 164, accounting for 5.63% and 24.33% of the OTUs, respectively. Results from the data indicated that WV treatment increased the composition of dominant bacterial and fungal genera in the rhizosphere soil and formed a more diverse microbial environment in the bacterial community structure, thereby benefiting the growth of cherry radishes. After WV treatment, there were significant differences in the relative abundance of bacterial communities at both the phylum and genus levels (Fig. 5). After 200-fold diluted WV treatment, the proportion of Actinobacteriota and Firmicutes in soil samples increased, with respective



225	increases of 40.88% and 126.67% compared to the CK group. Actinobacteriota can promote the
226	decomposition of soil organic matter and nutrient transformation, thereby contributing to the
227	improvement of soil fertility (Lan et al. 2022; Ren et al. 2018). The WV compounds contained
228	68.21% organic matter, which stimulated the activity of actinomyces and thereby increased its
229	abundance. In addition, Actinobacteriota also had a certain bioprotective effect, thus inhibiting the
230	growth of pathogenic bacteria and promoting the healthy growth of cherry radish. Meanwhile, the
231	abundance of <i>Proteobacteria</i> (up to 25.82%) in high carbon soil was promoted by W200
232	application (Fierer et al. 2007), and it was considered as an important soil symbiont together with
233	Firmicutes (Anderson et al. 2018; Zhalnina et al. 2015). Moreover, the bacterial genera with
234	relative abundance greater than 1% in soil treated with WV at different dilution concentrations and
235	CK were Arthrobacter, Sphingomonas, Vicinamibacterales, Vicinamibacteraceae, Massilia,
236	g_RB41, norank_f_norank_o_norank_c_KD4-96, and norank_f_Roseiflexaceae (Fig. 5b).
237	More importantly, the abundance of Arthrobacter, Sphingomonas, and norank_f
238	norank_o_Cyanobacteriales genera in WV at a concentration of 200 times was significantly
239	higher (over 10%) than other treatments, indicating a significant change in the proportion of
240	dominant bacterial genera in the rhizosphere soil of radish. Especially for norank_f
241	norank_o_Cyanobacteriales, which were between 15.34 and 230.05 times higher than CK and
242	W400. Notably, WV additives stimulate radish to absorb rhizosphere elements, degrade variety
243	aromatic compounds (the main component of WV), and drive nitrogen cycling by increasing the
244	abundance of dominant soil microorganisms, such as Arthrobacter (Schwabe et al. 2021),
245	Sphingomonas (Gong et al. 2016; Liu et al. 2017), and norank_f norank_oCyanobacteriales
246	(Wang et al. 2023).
247	Additionally, compared to CK and W400 treatments, the fungi in the W200 treatment group
248	showed a significant increase (0.98-25.06 fold higher) in the main phylum, specifically
249	Basidiomycota, unclassified_k_Fungi, and Blastocladiomycota, with the main fungal genera
250	being unclassified_f_Stachybotryaceae. Basidiomycota, as a crucial decomposer, generates
251	enzymes (peroxide) that enzymatically break down plant components such as cellulose and lignin,



thereby augmenting the overall carbon pool of the soil (Ahmed et al. 2020). Moreover, *unclassified\_k\_Fungi*, as a dominant genus in rhizosphere soil, stimulates fungi that generate total nitrogen (Yang et al. 2022). The organic matter (W200) in the rhizosphere promotes the abundance of *Blastocladiomycota* (Zhang et al. 2021), which is beneficial for crop growth. Similarly, *Stachybotryaceae* also showed a positive response to nitrogen in the rhizosphere soil (Kumar et al. 2022). These results also indicated that fungal communities displayed a vital role in the utilization and absorption of soil nutrients by crops.

#### 3.5 The Relationship between Radish Growth Factors and Microbial Communities

The correlation plot generated in Fig. 6 and S5 could dentify the microbial communities and 260 growth factors mediating the promotion of radish growth by WV. The root growth indicators 261 (length, tip, surface area, and diameter) showed significant correlation (p < 0.05) with the genera of 262 Arthrobacter, norank f norank o Cyanobacteriales, and Agromyces (Fig. S5). Among them, 263 the Arthrobacter and norank f norank o Cyanobacteriales in the W200 treatment group were 264 1.85-297.85 times higher than those in the CK and W400 treatment groups. It has been reported 265 that Arthrobacter (Schwabe et al. 2021) and norank f norank o Cyanobacteriales (Wang et 266 al. 2023) degrade aromatic compounds (WV) to drive the cycling of nitrogen nutrients in the 267 rhizosphere. Meanwhile, norank f norank o Cyanobacteriales, Ellin6067, and Agronomyces 268 exhibit a significant relationship (p<0.05) with photosynthesis. In the soil treated with W200, it 269 was found that norank f norank o Cyanobacteriales(Wang et al. 2023) and Agronomyces 270 271 (Wang et al. 2020) were involved in nitrogen and carbon cycling, respectively, thereby promoting 272 radish growth and significantly improving physiological indicators. For fungal communities, Allomyces, Clonostachys, Fusarium, Fusicolla, Knufia, Nigrospora, 273 Paraconiothyrium, Preussia, Talaromyces, Trichocladium, unclassified f Didymellaceae, 274 unclassified f Stachybotryaceae, unclassified Mortierellomycota showed a positive response 275 (p<0.05) to root length, root number, and root surface area of radish, while also showed a positive 276 correlation (p < 0.05) with aboveground biomass and fruit (Fig. 6). The fungi that positively 277 correlated (p<0.05) with the photosynthesis (except for Ci) of radish include Albifimbria, 278



Allomyces, Calcarisporiella, Clonostachys, Fusarium, Fusicolla, Knufia, Nigrospora, 279 Paraconiothyrium, Preussia, Talaromyces, unclassified c Sordariomycetes, 280 281 unclassified Chytridiomycota, unclassified f Didymellaceae, 282 unclassified f Stachybotryaceae, unclassified Mortierellomycota. Particularly, Allomyces, Fusarium, Fusicolla, Trichocladium, and unclassified f Didymellaceae, showed a very 283 significant correlation with radish growth and physiological indicators (p<0.01). The common 284 285 bacterial species (such as, Cladorrhinum (Barrera et al. 2019), Fusarium (Yuan et al. 2020), Allomyces (Zhang et al. 2013), Clonostachys (Fournier et al. 2020)) in the soil could decompose 286 the organic matter (WV), or WV could activate these bacteria to enhance the absorption of 287 nutrients by radish, thus promoting the development of its root system and ultimately boosting 288 crop photosynthesis and yield. Studies have already verified that Fusarium and Fusarium were the 289 290 main rhizosphere populations that promoted rapeseed growth and resulted in a higher yield (Lay et al. et al. 2018). Data showed that WV stimulated the dominant microorganisms in the radish 291 rhizosphere, thus increasing nutrient absorption and boosting yield. 292

#### 4. Conclusions

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In this study, the effects of wood vinegar on physiological indicators and rhizosphere 294 microbial community of cherry radish were studied through soil culture experiments. The 295 experimental results indicated that within a certain concentration range (W50-W200), cherry 296 297 radish yield gradually increased with the increasing wood vinegar concentration. When the 298 concentration of added wood vinegar reached W200, the diameter, biomass, and yield of radish 299 increased by 42.43%, 34.08%, and 44.91%, respectively. After applying wood vinegar (W200), the maximum net photosynthetic rate of the leafy cherry radish reached 25.53 µm CO<sub>2</sub>/(m<sup>2</sup>·s). 300 which showed the same growth trend as other photosynthetic indicators. The application of wood 301 vinegar is beneficial for the growth and reproduction of microbial communities, and the 302 microorganisms (e.g., Allomyces, Fusarium, and Fusicolla) have a very significant impact 303 (p<0.01) on promoting the growth of cherry radish. The promoting effect may be related to the 304 enhanced soil microbial activity by wood vinegar, or through promoting the absorption of 305



306	nutrients. Considering the types of biomasses and pyrolysis temperature varied largely, further
307	information about the wood vinegar on the growth and yield of cherry radish, even many other
308	plants, needed further conducted.
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315	Conflicts of interest
316	The authors declare no conflicts of interest.
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324	Author Contributions
325	Shiguo Gu, Wei Zhu, Xiaokang Li, and Yongkang Niu: writing-original draft, formal analysis,
326	conceptualization, and methodology. Liying Ren and Qingshan He: conceptualization and
327	resources. Yuying Ren: resources and investigation. Shiguo Gu: project administration. Shiguo



Gu, Binbin Sun and Qingshan He: funding acquisition, supervision, and writing—review & editing.



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Fig. 1. Plots of carbon (CHO, CHON, CHONS, and CHOS chemicals)

(a) and oxygen (CHO, CHON, CHONS, and CHOS chemicals) (b) number versus DBE of WV. (c) Van Krevelen diagrams of CHO, CHON, CHONS, and CHOS chemicals for biomass pyrolysis derived-WV, and (d) Bar diagrams for the contribution of the major classes for biomass pyrolysis-derived WV.

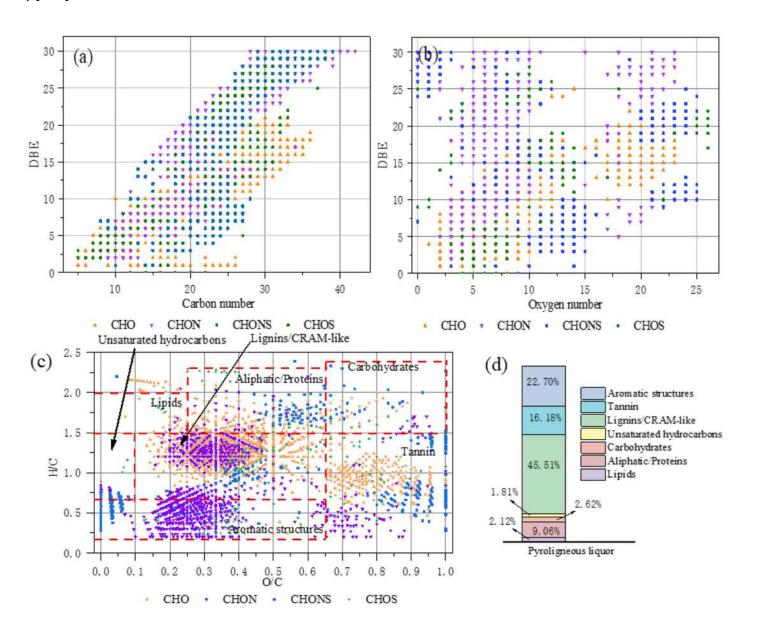




Fig. 2 Effect of wood vinegar treatment on the diameter (a) and weight (b) of cherry radish fruit. Data with different letters are significantly different (p < 0.05).

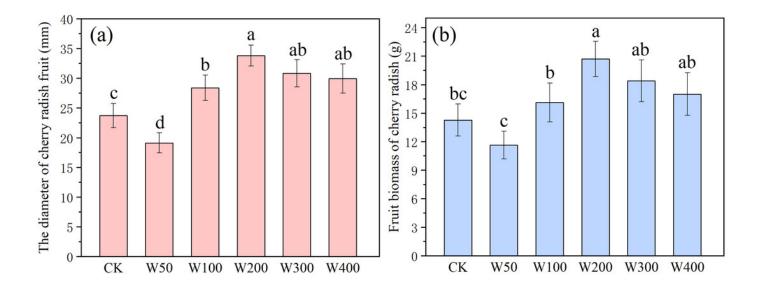




Fig. 3 The gas exchange parameters: (a) net photosynthetic rate (Pn), (b) transpiration rate (Tr), (c) stomatal conductance (Gs), and (d) intercellular  $CO_2$  concentration (Ci) of cherry radish.

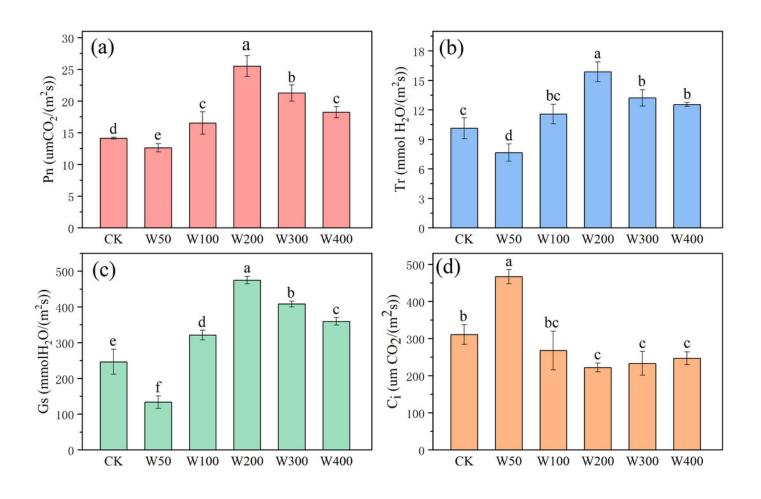




Fig. 4 Effects of Wood Vinegar on the Abundance and Diversity of Bacteria (a) and Fungi (b) in Rhizosphere Soil

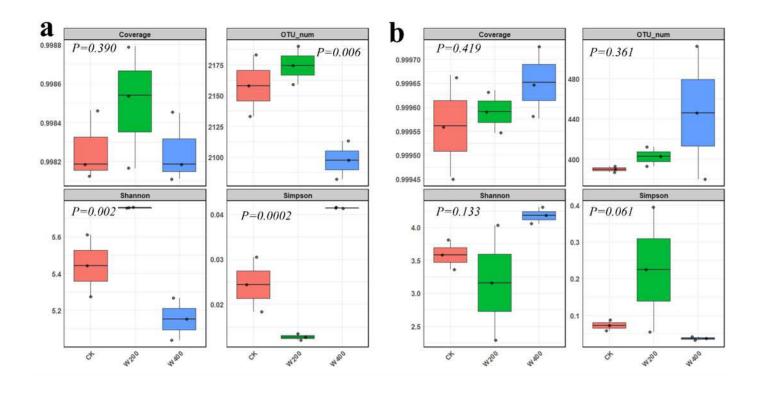




Fig. 5 The relative abundance of soil bacteria (phylum (a) and genus (b)) and fungi (phylum (c) and genus (d))

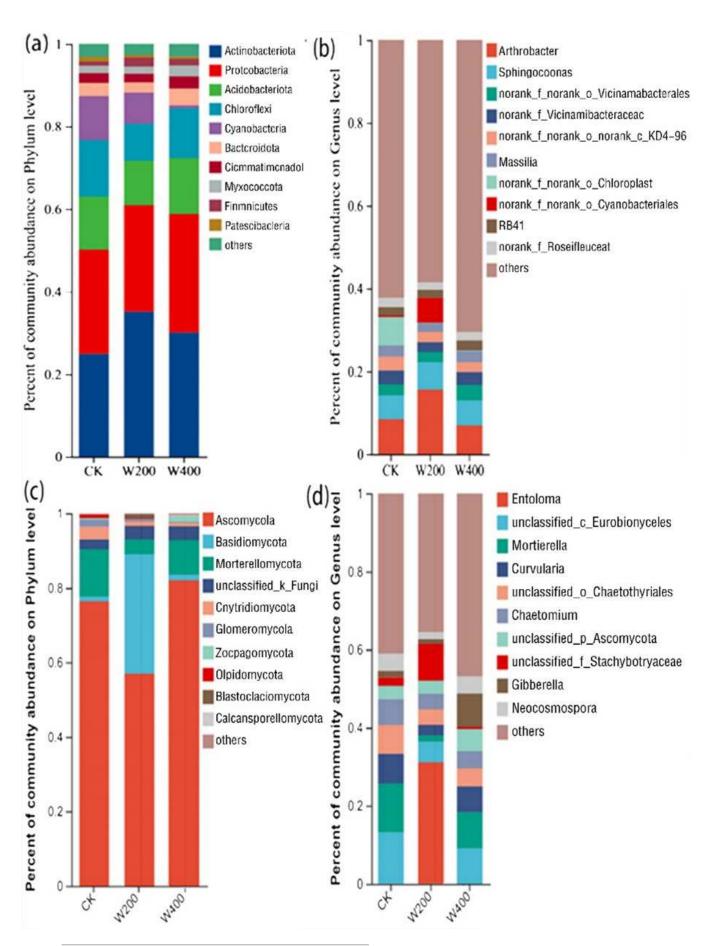




Fig. 6 Thermogram analysis of the correlation between fungi community and growth factors. Note: \* Significant correlation at 0.05 level, \* \* Very significant correlation at 0.01 level.

