

1 *Article*

2 **Regulatory Effects of Melatonin on *Hemerocallis fulva* Growth, Soil Physicochemical**
3 **Properties and Rhizosphere Bacterial Communities under Cadmium Stress**

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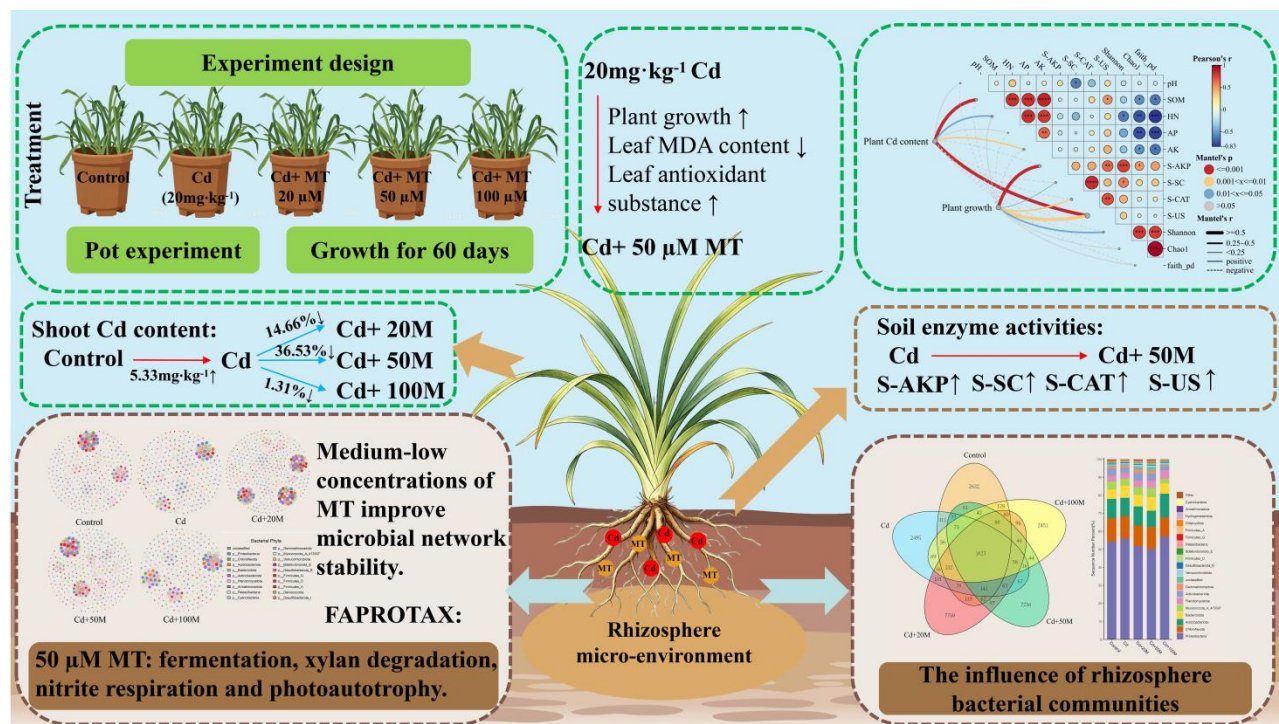
8

9 Abstract

10 *Hemerocallis fulva* (daylily), native to Asia, is valued for its ornamental, medicinal, and cultural uses.
11 Cadmium (Cd) stress limits growth of daylily. This study evaluated effects of exogenous melatonin
12 (MT) on daylily growth, physiology, soil properties, and rhizosphere bacterial communities under Cd
13 stress. One-year-old seedlings were treated with Cd alone ($20 \text{ mg} \cdot \text{kg}^{-1}$) or Cd combined with 20, 50,
14 or 100 μM MT. 50 μM MT showed the strongest alleviating effect, increasing plant height by 63.36%,
15 shoot fresh and dry weights by 59.90% and 72.04%, root fresh and dry weights by 72.63% and
16 68.72%, chlorophyll content by 46.15%, and soluble sugar content by 32.18%. It also increased SOD,
17 CAT, and POD activities by 112.02%, 26.82%, and 69.91%, respectively, and reduced shoot Cd
18 accumulation. Soil enzyme activities improved, with S-AKP, S-SC, S-CAT, and S-US increasing by
19 7.47%, 39.22%, 48.75%, and 13.86%, respectively. Bacterial richness was enhanced, and 50 μM MT
20 yielded 9172 total ASVs and 7236 unique ASVs. The rhizosphere bacterial community composition
21 changed, reflected in elevated abundances of Chlamydia, Firmicutes A, Deinococcota and the
22 genus *Chryseobacterium*. Network analysis indicated a more complex bacterial co-occurrence
23 pattern under 50 μM MT treatment, and FAPROTAX predicted higher abundances of functions
24 related to fermentation, xylan degradation, nitrite respiration, and photoheterotrophy. PLS-PM
25 indicated that shoot Cd content and soil AK were associated with bacterial richness, while bacterial
26 richness, S-SC activity, and shoot Cd content were associated with plant growth.

27 **Keywords:** Bacterial community structure; Cd contamination; *Hemerocallis fulva* seedlings;
28 melatonin; soil physicochemical properties

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31

32 **1. Introduction**

33 Cadmium (Cd) is highly toxic, mobile, difficult to degrade, and prone to bioaccumulation,
 34 making it a major environmental stressor that threatens soil ecological safety and inhibits plant
 35 growth (Kumar et al., 2023). Cd stress can severely disrupt physiological processes, inhibiting
 36 chlorophyll synthesis and photosynthesis, which reduces photosynthetic efficiency (Zhu et al., 2021).
 37 It can also impair nitrogen, phosphorus, and potassium uptake and transport, disturb nutrient balance,
 38 and inhibit cell division (Kaushik et al., 2024).

39 Cd contamination also impairs soil ecosystem health (Guo et al., 2024). Once introduced into soil,
 40 Cd can bind to organic matter and clay minerals, altering aggregate stability and reducing porosity
 41 and permeability (Manzoor et al., 2025). It further inhibits soil enzyme activity and nutrient
 42 mineralization. Gao et al. (2025) found that Cd contamination disrupts the soil phosphorus (P) cycle
 43 and significantly reduces the activity of soil alkaline phosphatase (AKP) and acid phosphatase (ACP).
 44 Various remediation approaches have been proposed to reduce heavy-metal mobility and toxicity.
 45 For example, red mud particles can effectively adsorb Pb²⁺, Cd²⁺, and Cu²⁺, with the adsorption

capacity generally following the order $\text{Pb}^{2+} > \text{Cd}^{2+} > \text{Cu}^{2+}$ (Bai et al., 2022). In addition, red mud and fly ash can be converted into high-strength geopolymers, providing a potential route for the comprehensive utilization of industrial waste and sustainable environmental management (Bai et al., 2023). Recovered phosphorus-bearing minerals, such as MAP, KMP, and HAP, have been reported to reduce Cd mobility and improve soil pH, urease activity, and bacterial Shannon diversity in contaminated soil (Qiu et al., 2024). More broadly, integrated soil management strategies, including water regulation, soil carbon restoration, cropping diversification, and rhizosphere engineering, are important for improving ecosystem resilience and sustainability (Wang et al., 2025a). Soil microorganisms are closely involved in nutrient cycling and aggregate formation and are essential for maintaining soil functions (Kumar et al., 2023). Cd directly alters microbial habitats and metabolism (Elrys et al., 2025). As a strong selective pressure, Cd causes tolerant groups to gradually gain the upper hand, while sensitive groups are eliminated, thereby undermining the diversity and stability of the community (Xiao et al., 2025). With these changes in community composition, the microorganisms' ability to convert carbon and nitrogen, along with their capacity to promote plant growth, will also decline (Shao et al., 2023). Microbial imbalance caused by Cd pollution disrupts this interactive network, further reducing plants' tolerance to Cd stress and ultimately creating a vicious cycle of increased pollution, functional decline, and reduced resistance (Zhu et al., 2024). Beneficial plant-microbe interactions, particularly arbuscular mycorrhizal symbioses, can enhance nutrient acquisition, improve resistance to abiotic stress, and contribute to soil structure maintenance through hyphal networks (Wang et al., 2025b). Therefore, regulating the rhizosphere microbial community may represent an important strategy for improving plant adaptation to Cd stress.

Daylily is an important plant resource with both ornamental and medicinal value. Its vibrant and varied flower colors make it widely used in landscaping and cut-flower production, giving it significant economic and aesthetic worth. In traditional medicine, the roots, stems, leaves, and flowers of daylilies can be used for medicinal purposes because they are rich in various bioactive compounds with anti-inflammatory and antioxidant properties (Hsu et al., 2023). Recent multi-omics

72 research on rare medicinal fungi has further demonstrated that developmental processes can be
73 closely associated with the biosynthesis of bioactive compounds (Jia et al., 2026). Soil Cd
74 contamination seriously compromises the growth and quality of daylily (Qadir et al., 2014). Cd
75 stress causes fluctuations or decreases in the content of medicinal active ingredients, affecting the
76 stability and safety of their medicinal properties.

77 Melatonin (MT) is a naturally occurring indoleamine. In plants, it is widely recognized as a
78 multifunctional endogenous signaling molecule involved in root growth, fruit development,
79 flowering, senescence, and various stress responses (Zhang et al., 2024). Arnao et al. (2018) reviewed
80 the roles of MT in plant physiology and noted that exogenous MT can improve stress tolerance
81 through multiple mechanisms, including activation of antioxidant defenses, removal of excessive
82 ROS, preservation of membrane stability, and modulation of hormone signaling pathways. Similar
83 cue-dependent metabolic reprogramming has also been observed in microalgae, where IAA
84 combined with high nitrogen promoted lutein accumulation in *Chlorella protothecoides* by enhancing
85 TCA cycle activity and redirecting carbon flux toward carotenoid biosynthesis (Li et al., 2026). MT
86 can also regulate the expression of genes involved in Cd absorption and compartmentalization,
87 reducing the toxicity of Cd to photosynthetic organs (Amjadi et al., 2021). Plant tolerance to stress
88 also depends on the rhizosphere microenvironment. Du et al. (2022) found that MT may enhance
89 nitrogen uptake by altering the abundance of specific microbial populations, thereby improving plant
90 stress tolerance. In the study on barley under low-temperature stress, exogenous MT treatment
91 significantly enriched beneficial microbial taxa such as Proteobacteria and Acidobacteria in the
92 rhizosphere, improved nutrient transformation efficiency, and enhanced the plant's nutrient use
93 efficiency and stress tolerance (Jiang et al., 2022).

94 To investigate the effects of MT on the growth of daylilies and soil health under Cd stress, we
95 conducted pot experiments using the daylily cultivar 'Golden Doll'. The study encompassed
96 measurements of plant growth indices, physicochemical properties of rhizosphere soil, and enzyme
97 activity, as well as analysis of bacterial community structure based on 16S rRNA sequencing, with

the aim of providing scientific guidance for the application of MT in the remediation of heavy metal-contaminated soils.

2. Materials and Methods

2.1 Test Materials

The test plants were daylily seedlings (*Hemerocallis fulva* ‘Golden Doll’) of uniform growth (about 10-12 cm tall), each with six leaves, purchased from China Chunyi flower horticulture company. The experiment was conducted in a greenhouse (119°56'26.6"E, 32°28'33.4"N) under natural light (air temperature: 25°C, relative humidity: 60%). During the entire experiment, each pot was randomly relocated five times to even out environmental conditions. The initial physicochemical properties of the soil are shown in Table 1.

Table 1. The physical and chemical of fresh sandy loam soil.

Indicators	Content
pH	6.97
Organic matter	16.9 g·kg ⁻¹
Total nitrogen	1.2 g·kg ⁻¹
Total phosphorus	0.4 g·kg ⁻¹
Total potassium	15.4 g·kg ⁻¹
Available nitrogen	0.18 g·kg ⁻¹
Available phosphorus	0.015 g·kg ⁻¹
Available potassium	0.063 g·kg ⁻¹
Cd	0.41 mg·kg ⁻¹

Soil pH, organic matter (SOM), total phosphorus (TP), total potassium (TK), available phosphorus (AP), and available potassium (AK) were measured according to the agricultural industry standards of the People’s Republic of China. Total nitrogen and available nitrogen were determined according to the Forestry Industry Standard of the People’s Republic of China LY/T 1228-2015.

2.2 Experimental Design

114 Based on previous studies on heavy metal Cd (Chen et al., 2025) and MT (Zhang et al., 2024),
115 five treatments were established: Control (no Cd, no MT), Cd (20 mg·kg⁻¹ Cd), and Cd combined
116 with MT at 20, 50, and 100 μM, respectively. CdCl₂·2.5H₂O served as the Cd contamination. We
117 thoroughly mixed the pollution sources with the soil (perlite to soil ratio of 1:2) and let the mixture
118 stand for two weeks before starting the planting experiments. For each treatment, three replicate pots
119 were prepared. Each plastic pot was filled with 2 kg of potting mix and planted with six healthy
120 daylily seedlings. One week after planting, the Cd + MT group was uniformly irrigated with a MT
121 solution at a specific concentration, which was applied to the rhizosphere surface of each pot. This
122 MT application was repeated three times at ten-day intervals, with 200 mL per pot each time. Routine
123 greenhouse management was maintained throughout the experiment.

124 *2.3 Determination of growth indicators*

125 Growth indicators were measured 60 days after the final MT application. Plant height was
126 assessed from the soil surface to the tip of the tallest leaf. After the plants were carefully dug out,
127 rhizosphere soil was sampled first, followed by collection of the plants themselves. The collected
128 plants were then rinsed thoroughly under running tap water and washed three times with ultrapure
129 water. After removing surface moisture, each plant was divided into shoots and roots. We then
130 determined the fresh and dry biomass of both organs using the method described by Zhang et al
131 (2022). The soluble sugar content was measured using the anthrone colorimetric method (Hu et al.,
132 2017). Chlorophyll content was measured spectrophotometrically after extraction with 80% acetone
133 (Lichtenthaler, 1987).

134 To determine malondialdehyde (MDA) content in daylily leaves, we adopted the TBA method
135 (Song et al., 2011). Superoxide dismutase (SOD) activity in daylily leaves was measured using the
136 nitroblue tetrazolium (NBT) method (Dhindsa, 1981). Catalase (CAT) activity was determined by
137 ultraviolet spectrophotometry (Li et al., 2023b). Peroxidase (POD) activity was assayed using the
138 guaiacol method (Maehly and Chance, 1954).

The Cd content in the shoot and root of daylily plants was determined by nitric acid-hydrogen peroxide-hydrofluoric acid multi-acid digestion combined with ICP-MS.

2.4 Soil sample collection

After the final MT was applied for 60 days, plant samples were collected and soil was sampled. We gently shook the roots to let loose soil fall off, leaving only the thin layer within about 2 mm of the root surface. Subsequently, the roots were placed into sterile 250 mL Erlenmeyer flasks containing 50 mL of sterile distilled water and shaken at 180 rpm for 30 minutes. This process was repeated three times. The resulting turbid suspension was collected and centrifuged twice to form a dense pellet, which was used as the rhizosphere soil sample.

2.5 Determination of soil enzyme activity

Soil sucrase (S-SC) and catalase (S-CAT) activities were determined according to the colorimetric procedure described by Du et al. (2018). S-AKP activity was measured by a colorimetric method using disodium phenyl phosphate as the substrate. The urease (S-US) assay is performed using the indophenol blue colorimetric method.

2.6 Determination of rhizosphere microbes

DNA from soil microorganisms was extracted following the CTAB protocol (Lohnoo et al., 2014). The V4-V5 region of the 16S rRNA gene was amplified with primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3') for bacterial community analysis. After library quality was evaluated using an Agilent 5400 system (Agilent Technologies, USA), 250-bp paired-end sequencing was performed on an Illumina platform at Wekemo Tech Group Co., Ltd. (Shenzhen, China). Raw data were deposited in the NCBI database under project ID PRJNA1305099.

2.7 Data Statistics and Analysis

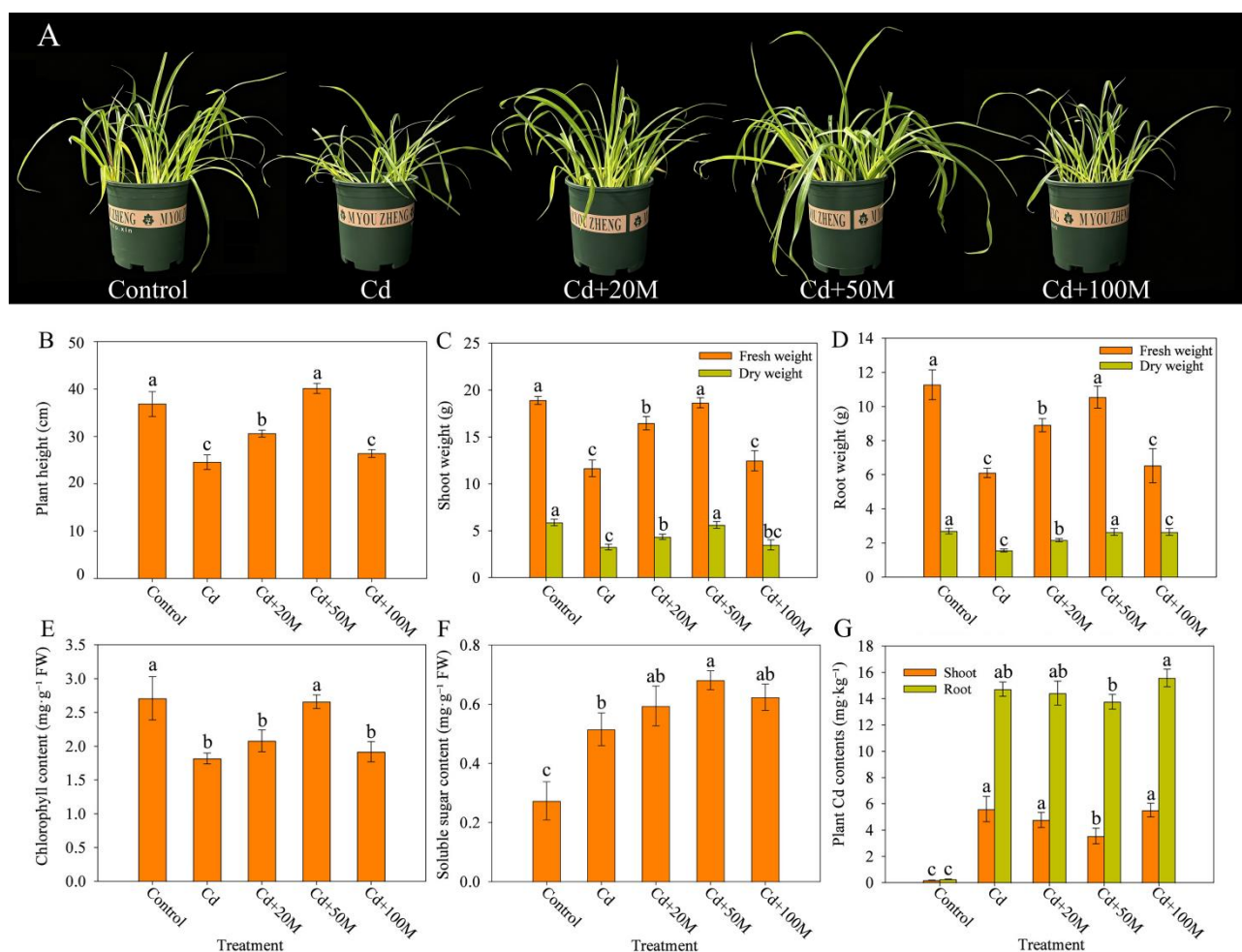
Bioincloud platform (<https://www.bioincloud.tech>) was employed to analyze the sequencing data (Gao et al., 2024). Data were analyzed using Excel 2019 and SPSS 24.0. One-way ANOVA followed by Duncan's multiple range test was applied at $P < 0.05$. Alpha-diversity indices were

165 calculated with the QIIME2 diversity plugin. NMDS based on Bray-Curtis distances was performed
166 using the metaMDS function in the vegan package of R (version 4.3.3). LEfSe analysis was
167 conducted with the microeco package in R ($LDA > 3$). The bacterial co-occurrence network was
168 constructed using the igraph package in R. Network topological visualization was realized using
169 Gephi (Version 0.9.2). Bacterial community functions were predicted using FAPROTAX software
170 (1.2.6). Spearman's rank correlation coefficient was used to assess relationships between variables.
171 P-values were adjusted for multiple testing using the FDR (Benjamini-Hochberg) method, with $q <$
172 0.05 considered significant. Partial least squares path modeling (PLS-PM) was conducted in R 4.4.1
173 using the plsrm package. Indicators with outer loadings < 0.70 were excluded. Model stability was
174 assessed by nonparametric bootstrapping with 1,000 resamples ($boot.val = TRUE$). Path coefficients
175 were considered significant when their 95% bootstrap confidence intervals excluded zero (Sanchez,
176 2013; Wang et al., 2026). Graphs were plotted using SigmaPlot 15.0 software.

178 3. Results

179 3.1 Seedling growth indices

180 Daylily seedlings grown under $20 \text{ mg} \cdot \text{kg}^{-1}$ Cd were noticeably stunted compared with the
181 controls (Figure 1A). Key growth parameters including plant height (Figure 1B), shoot weights
182 (Figure 1C), root weights (Figure 1D), and chlorophyll content (Figure 1F) were all markedly
183 declined. To test whether MT could mitigate Cd-induced growth reduction, we compared Cd-stressed
184 plants with those receiving Cd plus different MT concentrations. Under Cd stress, MT at $20 \mu\text{M}$ and
185 $50 \mu\text{M}$ partially reversed the adverse effects. The $50 \mu\text{M}$ MT treatment in particular enhanced several
186 growth traits: plant height by roughly 63.36% (Figure 1B); shoot fresh weight by about 59.90% and
187 shoot dry weight by 72.04% (Figure 1C); root fresh weight by about 72.63% and root dry weight by
188 68.72% (Figure 1D); chlorophyll content by about 46.15% (Figure 1E); and soluble sugar content by
189 32.18% (Figure 1F). By contrast, a higher concentration ($100 \mu\text{M}$) of MT did not exert any
190 growth-promoting effect on Cd-stressed daylilies.



191

192 **Figure 1.** Effects of exogenous MT on the growth indices and Cd accumulation of daylily seedlings
 193 under Cd stress. (A) The growth phenotypes. (B) Plant height. (C) Shoot weight. (D) Root weight.
 194 (E) Chlorophyll content. (F) Soluble sugar content. (G) Plant Cd content. Control: MT 0 μM + Cd 0
 195 $\text{mg}\cdot\text{kg}^{-1}$; Cd: Cd 20 $\text{mg}\cdot\text{kg}^{-1}$; Cd + 20M: Cd 20 $\text{mg}\cdot\text{kg}^{-1}$ + MT 20 μM ; Cd + 50M: Cd 20 $\text{mg}\cdot\text{kg}^{-1}$ +
 196 MT 50 μM ; Cd + 100M: Cd 20 $\text{mg}\cdot\text{kg}^{-1}$ + MT 100 μM . Error bars show the SD of three replicates.
 197 Different lowercase letters indicate significant differences at $P < 0.05$; subsequent figures follow the
 198 same notation.

199 3.2 Cd absorption

200 After harvest, we measured the Cd concentrations in the plants to assess their capacity for Cd
 201 uptake. Cd treatment markedly increased Cd content in the plants, and roots accumulated more Cd
 202 than shoots. This distribution follows the general rule of heavy metal translocation. Roots act as the
 203 first barrier, sequestering most soil Cd ions so that only a minor portion moves upward through

204 vascular tissues and Cd accumulates mainly in the roots. Compared with plants exposed to 20 mg kg⁻¹
205 Cd alone, the 50 µM MT treatment significantly reduced Cd concentrations in the shoots, while root
206 Cd accumulation did not differ significantly among treatments (Figure 1G).

207 3.3 Antioxidant system

208 Under 20 mg·kg⁻¹ Cd stress, the MDA content increased by 70.2%. Both 20 and 50 µM MT
209 reduced MDA levels, by 25.41% and 32.24%, respectively (Figure 2A). Cd stress reduced the
210 activities of SOD, CAT, and POD by 32.06%, 13.90%, and 11.28%, relative to the control. The
211 addition of 20 µM MT notably elevated enzyme activities: SOD by 57.10%, CAT by 19.19%, and
212 POD by 29.69%. A more pronounced enhancing effect was observed with 50 µM MT, which
213 increased SOD activity by 112.02%, CAT activity by 26.82%, and POD activity by 69.91% (Figure
214 2B–D). Under 100 µM MT treatment, SOD and POD activities in leaves did not differ significantly
215 from those in the Cd-only treatment, but CAT activity increased markedly.

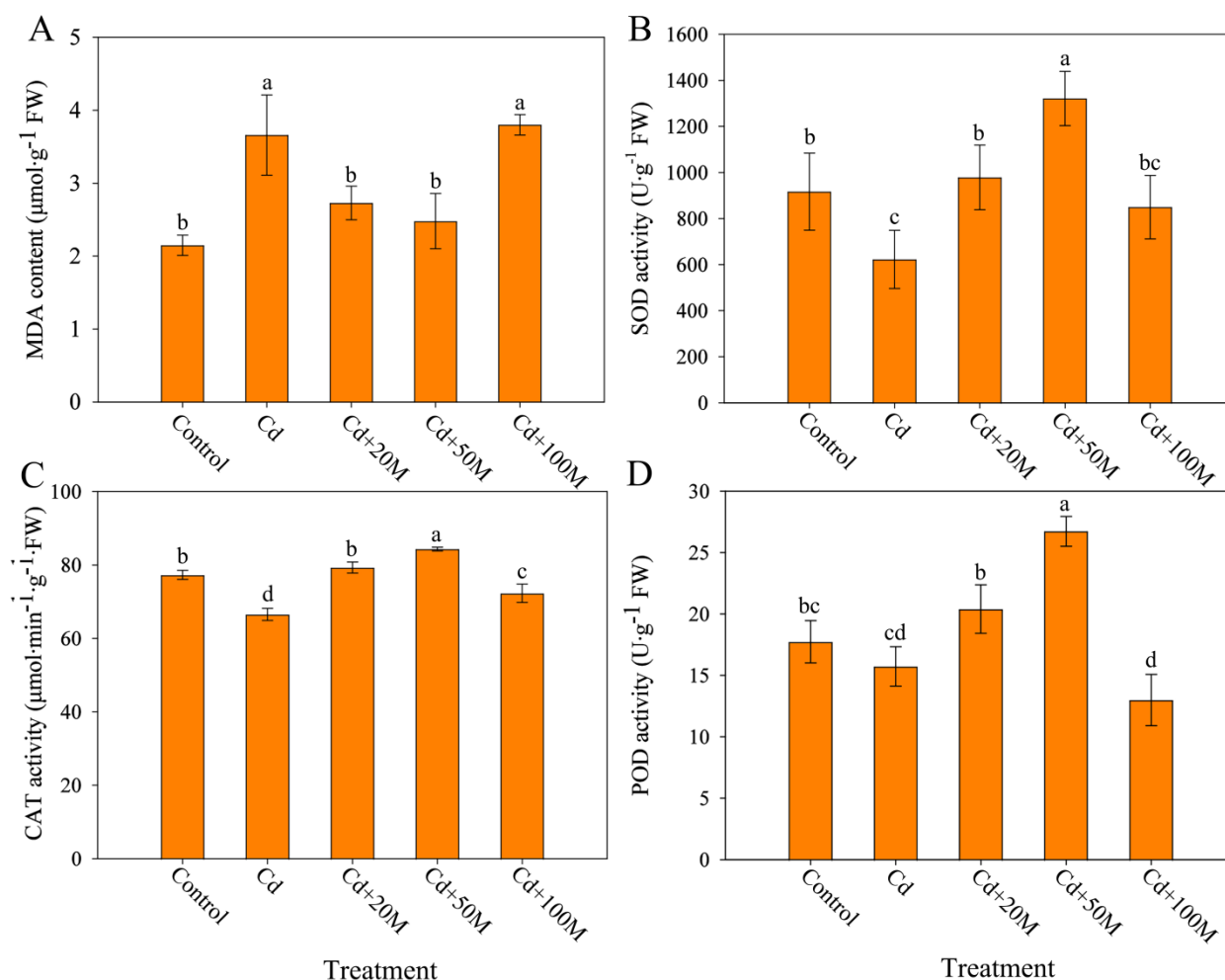
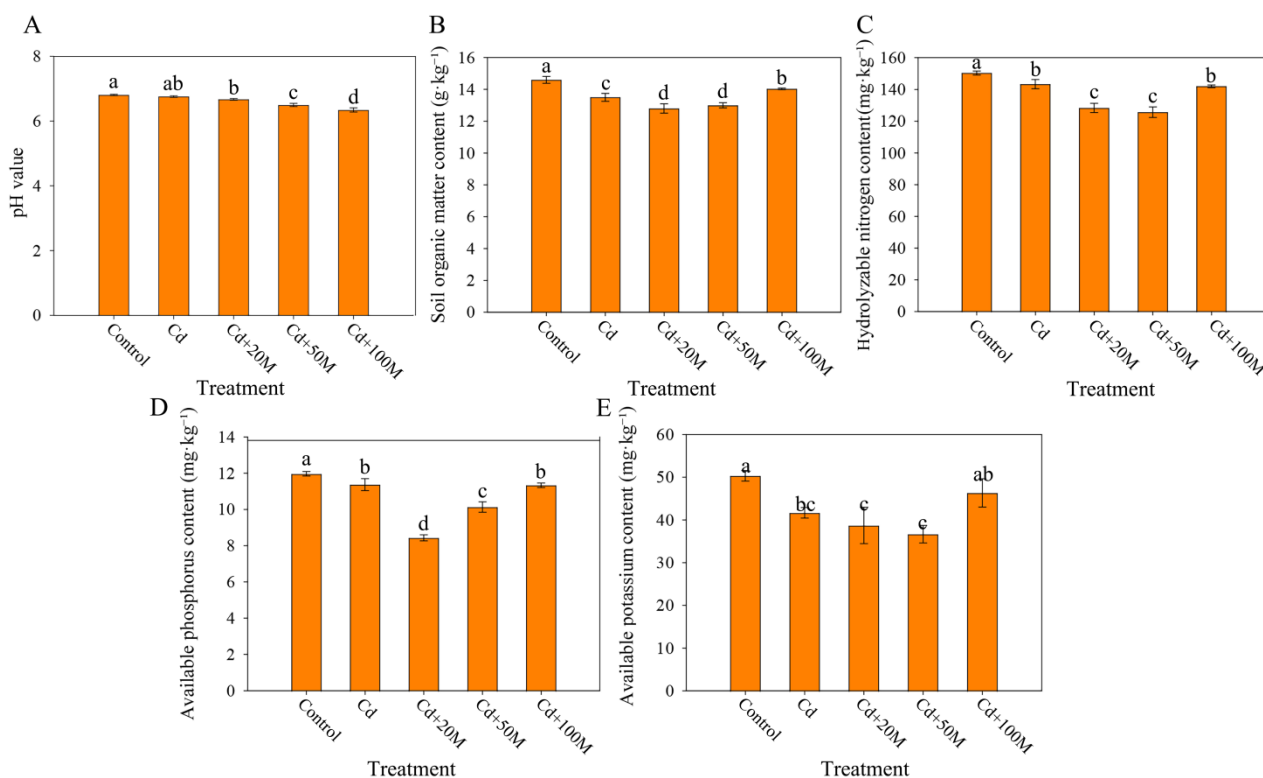


Figure 2. Effects of MT on MDA content and antioxidant enzyme in daylily leaves under Cd stress.

(A) MDA content. (B) SOD activity. (C) CAT activity. (D) POD activity.

3.4 Properties and enzyme activities of soil

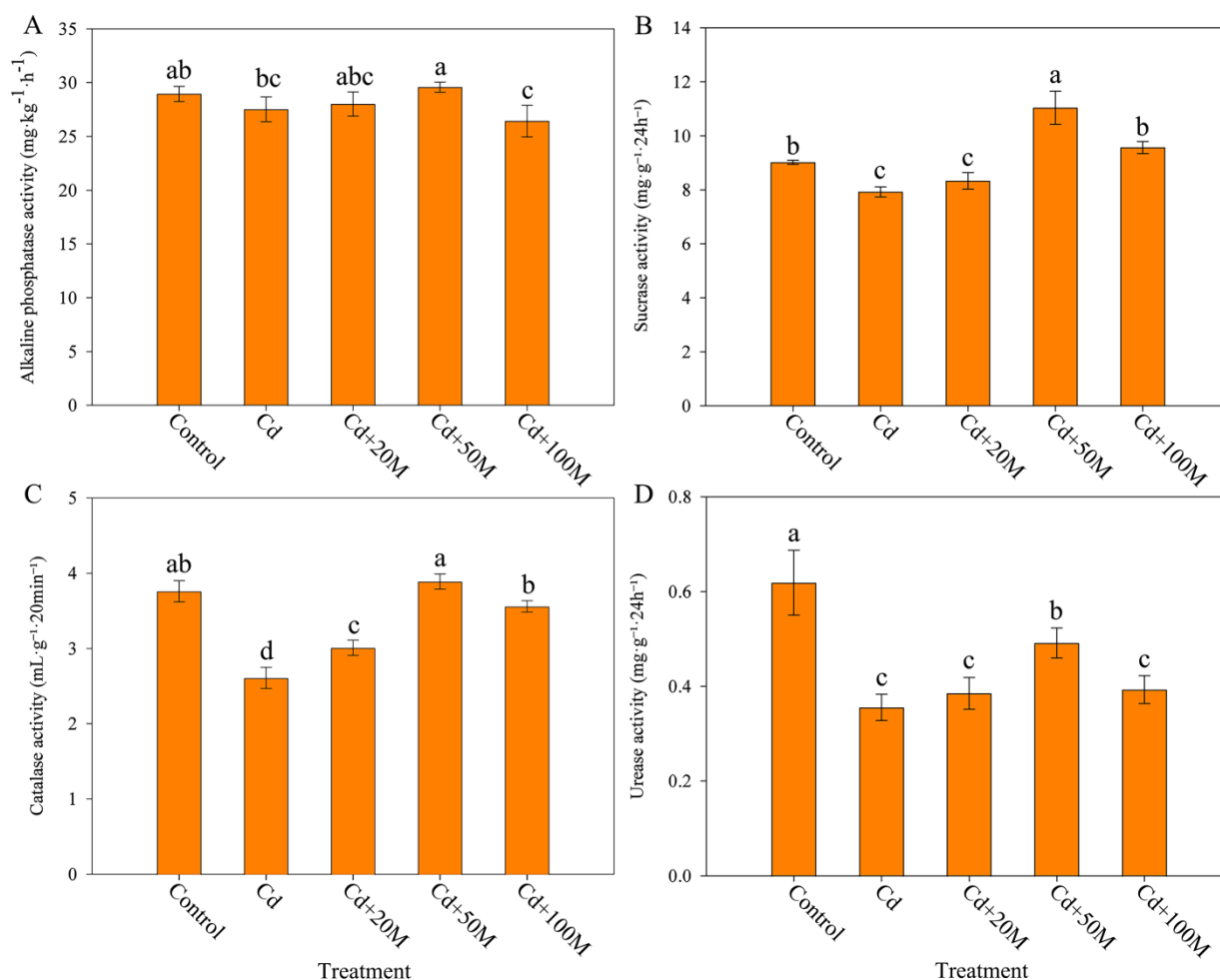
Soil pH decreased gradually as MT concentration increased. Under 20 $\text{mg}\cdot\text{kg}^{-1}$ Cd treatment, the contents of SOM, hydrolyzable nitrogen (HN), AK, and AP were all reduced compared with the control. Treatments with 20 μM and 50 μM MT further decreased these indicators, whereas soil organic matter, HN, AK, AP contents increased significantly under 100 μM MT treatment. This may be attributed to the better plant growth under 20 μM and 50 μM MT applications, which promoted greater nutrient uptake from the soil and consequently reduced the concentrations of these soil nutrients (Figure 3).



227

228 **Figure 3.** Effects of MT on soil physicochemical properties under Cd stress. (A) pH value. (B) SOM
229 content. (C) HN content. (D) AP content. (E) AK content.

230 Cd stress significantly inhibited S-SC, S-CAT, and S-US activities, and slightly reduced S-AKP
231 (Figure 4). Exogenous application of MT at different concentrations increased the S-SC, S-CAT and
232 S-US activities in Cd-contaminated soil to varying degrees, thereby alleviating the inhibitory effect of
233 Cd on soil enzyme activities. Across all treatments, 50 μM MT exhibited the optimal regulatory effect.
234 Compared with the Cd-stressed group, S-AKP activity increased by 7.47% (Figure 4A), S-SC activity
235 increased by 39.22% (Figure 4B), S-CAT activity increased by 48.75% (Figure 4C), and S-US
236 activity increased by 13.86% (Figure 4D).



237

238 **Figure 4.** Effects of MT on the activities of soil enzyme under Cd stress. (A) S-AKP activity. (B)
 239 S-SC activity . (C) S-CAT activity. (D) S-US activity.

240 3.5 Rhizosphere bacterial community structure

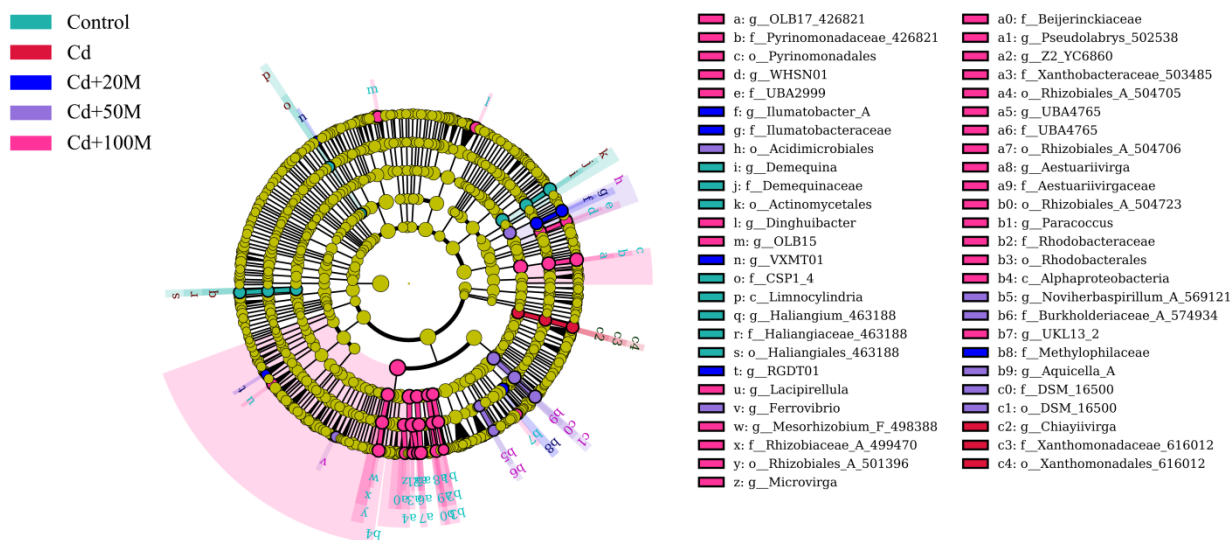
241 Figure 5A shows the shared and unique bacterial ASVs across different treatments, with 1023
 242 ASVs present in all groups. Compared with the control group (4902 total ASVs, 2632 unique ASVs),
 243 Cd stress reduced both total ASVs (4585) and unique ASVs (2495). In contrast to Cd stress without
 244 MT, the application of 20 μ M or 50 μ M MT led to a rise in both total and unique ASVs. Specifically,
 245 the 20 μ M MT treatment resulted in 10001 total ASVs and 7750 unique ASVs, while the 50 μ M MT
 246 treatment yielded 9172 total ASVs and 7236 unique ASVs. In contrast, the 100 μ M MT treatment
 247 exhibited the lowest total and unique ASV counts.

248 Figure 5B then shows the phylum-level relative abundances of bacterial communities.
 249 Proteobacteria, Chloroflexota, Acidobacteriota, Bacteroidota, Myxococcota_A_473307,

250 Planctomycetota, Actinobacteriota, and Gemmatimonadota predominated in all samples, each with a
251 relative abundance exceeding 1%. Proteobacteria was the most abundant phylum, accounting for
252 50.29%–57.12% of the community, followed by Chloroflexota (10.54%–12.91%) and
253 Acidobacteriota (8.99%–13.21%). In addition, the heatmap revealed distinct phylum-level
254 community structures among groups (Figure 5D). In the control group, Deinococcota and
255 Armatimonadota exhibited relatively higher abundance. In soils treated with 20 mg·kg⁻¹ Cd, the
256 characteristic phylum was Proteobacteria. Under 20 μM MT treatment, the characteristic phyla were
257 Gemmatimonadota and Patescibacteria. Under 50 μM MT treatment, Chlamydiota, Firmicutes_A,
258 and Deinococcota showed high abundance. At the highest concentration of MT (100 μM),
259 Acidobacteriota and Planctomycetota possessed relatively higher abundance. Compared with the 20
260 mg·kg⁻¹ Cd stress group, the 20 μM MT and 50 μM MT treatments significantly increased the
261 abundance of Patescibacteria.

262 The 20 most abundant genera in each group are presented in Figure 5C. *Aggregatilinea*
263 (3.6%~7.6%), *Devosia_A_501803* (2.3%~3.8%), *Thiobacillus* (0.09%~9.6%), *Phenylobacterium*
264 (1.4%~2.5%), *VBCG01* (1.3%~1.5%), *Chryseolinea* (0.78%~1.76%), *Bauldia* (0.93%~1.5%), and
265 *Usitatibacter* (0.96%~1.5%) were among the relatively abundant genera in the soil. The heatmap
266 revealed distinct genus-level community structures among groups (Figure 5E). In the control group,
267 *Aggregatilinea* and *Devosia_A_501803* exhibited relatively higher abundance. Under 20 mg·kg⁻¹ Cd
268 treatment, the characteristic genera were *JAAUUD01* and *Roseomonas_B*. Under 20 μM MT
269 treatment, the characteristic genus was *Thiobacillus*. Under 50 μM MT treatment, *Chryseolinea*
270 showed high abundance. At the highest concentration of MT (100 μM), *Arsenicitalea*,
271 *Phenylobacterium*, and *Alteraurantiacibacter* exhibited relatively higher abundance. Compared with
272 the 20 mg·kg⁻¹ Cd stress group, the 20 μM MT treatment reduced the abundances of
273 *Mesorhizobium_F_498388* and *Z2_YC6860*. Treatment with 100 μM MT led to a significant
274 reduction in the abundance of *Aggregatilinea*, while increasing the abundances of *Phenylobacterium*,
275 *OLB17_426821*, *Luteitalea*, and *Arsenicitalea*.

290 f_DSM_16500, g_Aquicella_A, o_Acidimicrobiales, f_Burkholderiaceae_A_574934,
 291 g_Noviherbaspirillum_A_569121 and g_Ferrovibrio. Some special ASVs increased in the soil
 292 under 100 μ M treatment including c_Alphaproteobacteria, o_Rhizobiales_A_504705,
 293 f_Xanthobacteraceae_503485, o_Pyrinomonadales, g_OLB17_426821,
 294 f_Pyrinomonadaceae_426821, o_Rhizobiales_A_504706, o_Rhizobiales_A_501396,
 295 o_Rhodobacterales, among others.



296
 297 **Figure 6.** LefSe analysis of daylily rhizosphere bacterial communities (Only LDA Score greater than
 298 3 are displayed).

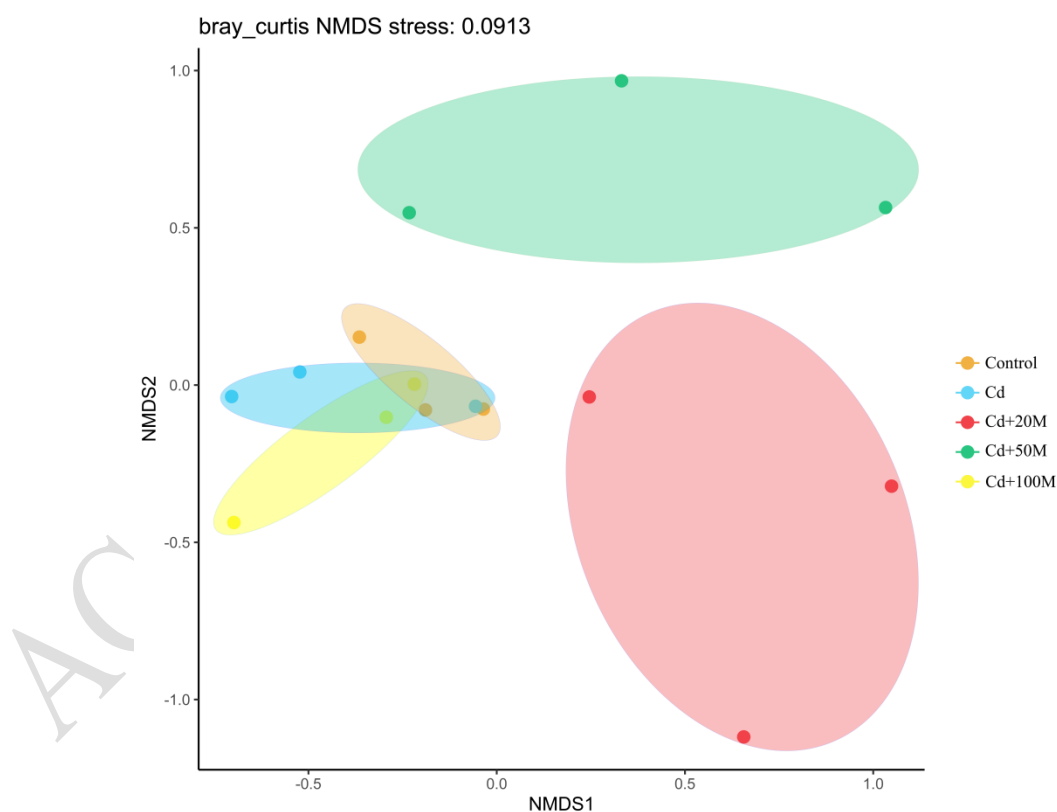
299 3.6 Diversity of soil rhizosphere microorganisms communities

300 To evaluate changes in alpha diversity of rhizosphere bacterial communities, we calculated the
 301 faith's phylogenetic diversity, Chao1, Shannon and Ace indices (Table 2). Exposure to 20 $\text{mg}\cdot\text{kg}^{-1}$ Cd
 302 stress overall reduced bacterial community richness and diversity. This indicates that Cd stress
 303 decreased both the richness and diversity of rhizosphere bacterial communities. Following the
 304 addition of 20 μ M and 50 μ M MT, all diversity indices increased markedly. However, 100 μ M MT
 305 did not alleviate the Cd-induced decline in bacterial diversity.

306 **Table 2:** Effects of MT on the α -diversity index of soil rhizosphere bacterial communities under Cd
 307 stress.

Treatment	faith's pd	Chao1	Shannon	Ace
Control	160±23b	2333±578bc	9.57±0.37ab	2341±578bc
Cd	147±27b	2074±668c	9.42±0.29b	2080±668c
Cd+20M	290±69a	4037±978a	9.82±0.46ab	4053±981a
Cd+50M	282±73a	3685±1165ab	10.16±0.27a	3699±1169ab
Cd+100M	145±11b	2046±187c	9.49±0.26b	2055±188c

308 Using Bray-Curtis NMDS based on ASVs and relative abundance, We found that MT treatment
309 significantly altered the rhizosphere bacterial community structure in daylily (Figure 7). NMDS
310 analysis showed that along the NMDS1 and NMDS2 axes, soil samples from the low and medium
311 MT treatments (20 μ M and 50 μ M) were clearly separated from those under Cd stress alone,
312 indicating changes in the structure of the bacterial communities.



313
314 **Figure 7.** NMDS analysis of the bacterial communities.

315 3.7 Rhizosphere bacterial co-occurrence network

316 We constructed co-occurrence networks based on soil samples from five treatments (Figure 8),
 317 with topological parameters listed in Table 3. 20 mg·kg⁻¹ Cd stress severely disrupted microbial
 318 interaction networks. The Cd treatment had 284 nodes and 500 edges, lower than 325 nodes and 926
 319 edges in the control group. Its average degree (3.521) and network density (0.012) were the lowest
 320 across all groups, indicating reduced interaction scale and tightness among microbial taxa under Cd
 321 stress. Positive correlations dominated networks of all treatments. MT application altered network
 322 structures obviously. 20 μM and 50 μM MT recovered network complexity effectively. Higher node
 323 number, edges number, average degree and network density were observed in these two groups.
 324 Average path length and network diameter both increased, pointing to a more complex hierarchy and
 325 greater resilience to disturbance. Under 100 μM MT, node number, edge number, and average degree
 326 all decreased. All treatments exhibited clustering coefficients greater than 0.6, a value characteristic
 327 of small-world networks. The Cd group showed the lowest value (0.012). MT treatments raised these
 328 coefficients and strengthened the local clustering patterns. Modality reached its peak (0.828) under
 329 Cd stress, then decreased following treatment with 20 μM and 50 μM MT, but rebounded to 0.800
 330 after treatment with 100 μM MT.

331 **Table 3:** Topological characteristic parameters of co-occurrence networks.

Treatment	Nodes number	Edges number	Average degree	Average path length	Network diameter	Network density	Clustering coefficient	Modularity
Control	325	926	5.698	2.521	9	0.018	0.625	0.687
Cd	284	500	3.521	2.285	9	0.012	0.606	0.828
Cd+20M	427	2035	9.532	7.126	18	0.022	0.714	0.775
Cd+50M	356	1155	6.489	4.692	11	0.018	0.639	0.735
Cd+100M	264	629	4.765	1.606	6	0.018	0.739	0.8

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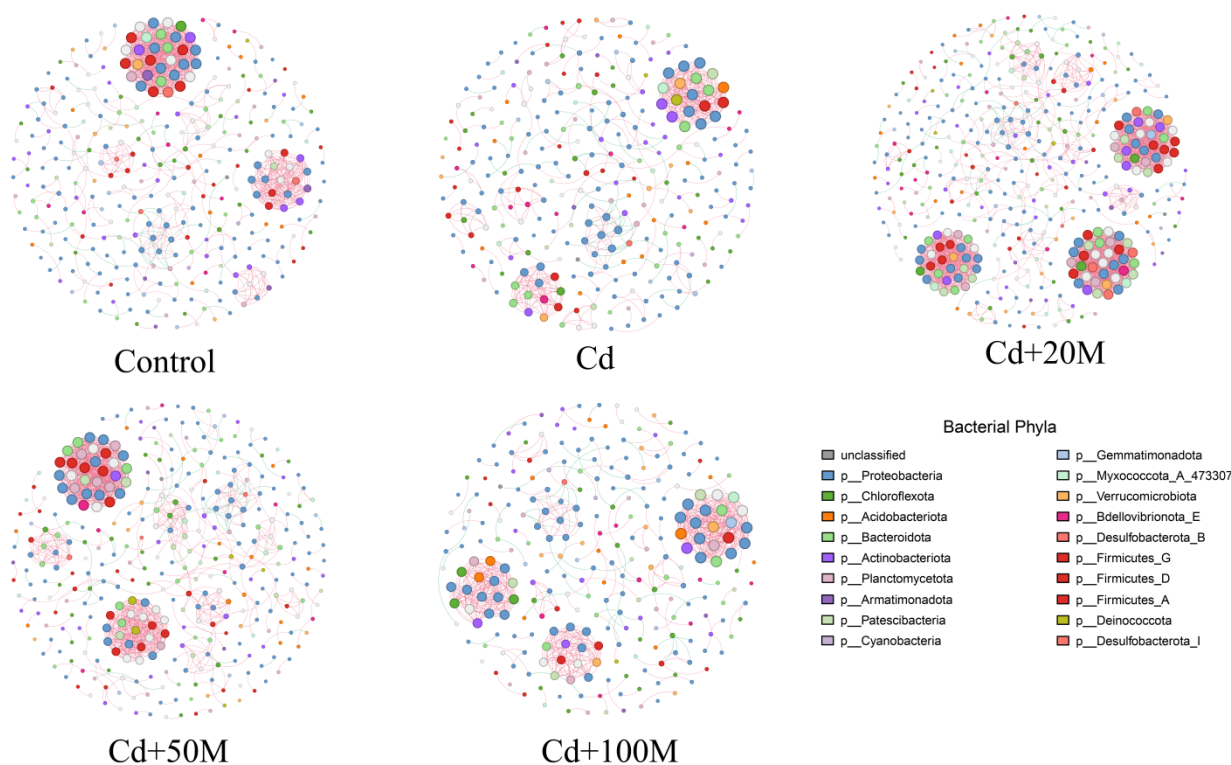
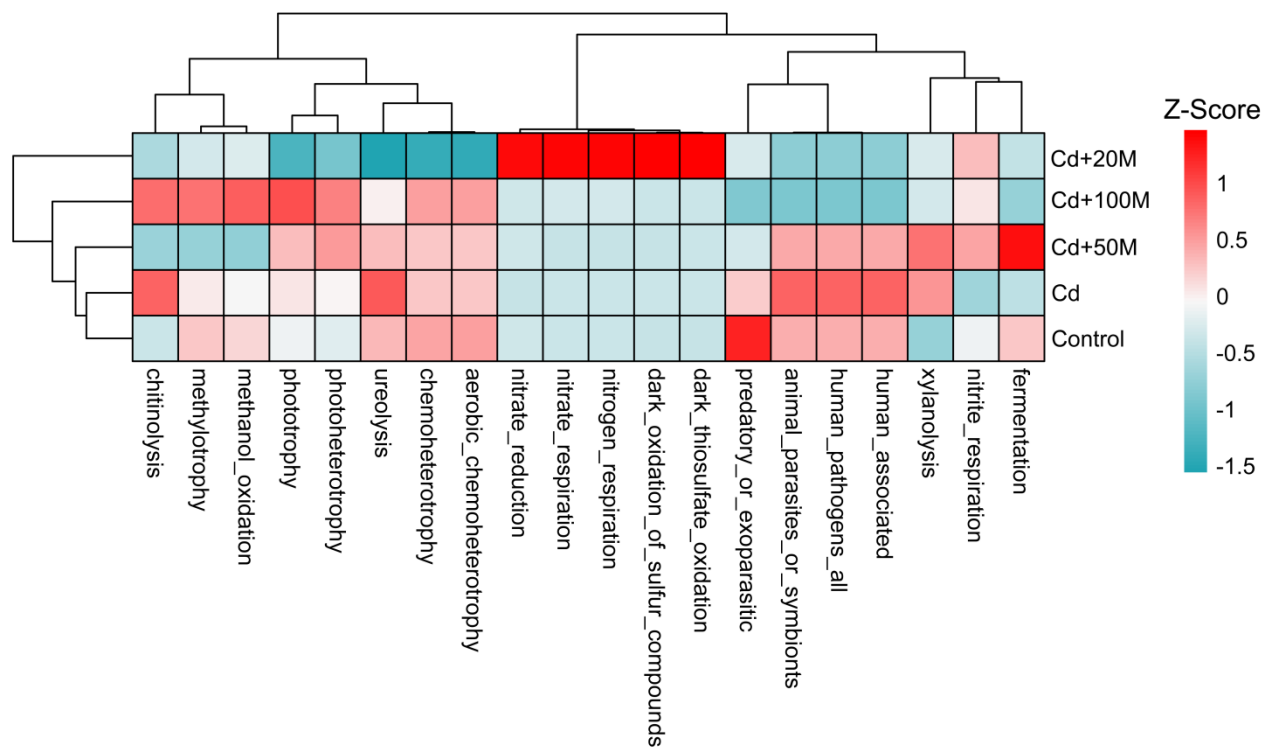


Figure 8. Co-occurrence network of rhizosphere bacterial communities. Nodes represent genera, with colors denoting different phyla. Solid lines show pairwise correlations: red for positive, green for negative relationships. Modules are highly interconnected genus clusters.

3.8 Function prediction analysis

Based on the taxonomic annotation results of 16S rRNA sequences, we used the FAPROTAX tool to predict the functions of the bacterial community. 66 functional types were obtained, and Figure 9 only shows the top 20 functional types in terms of relative abundance. In the control soil, the most abundant predicted functions were mainly related to predatory or exoparasitic, chemoheterotrophic, aerobic chemoheterotrophic, and human-associated microorganisms. Under Cd stress ($20 \text{ mg} \cdot \text{kg}^{-1}$), the relative abundance of functions related to ureolysis, chitinolysis, animal parasitism or symbiosis, and human pathogenicity increased markedly. $20 \text{ } \mu\text{M}$ MT was associated with higher predicted abundances of functions involved in dark oxidation of sulfur compounds, dark thiosulfate oxidation, nitrate respiration, nitrogen respiration, and nitrate reduction. The $50 \text{ } \mu\text{M}$ MT treatment was characterized by greater predicted abundances of fermentation, xylanolysis, nitrite

349 respiration, and photoheterotrophy. At 100 μ M MT, phototrophy, methylotrophy, and methanol
 350 oxidation became more prominent in the predicted functional profile.



351
 352 **Figure 9.** Bacterial functional prediction based on FAPROTAX.

353 *3.9 Correlation analysis*

354 The redundancy analysis (RDA) plot (Figure 10A) revealed significant differences in microbial
 355 community structure (permutation test, $P=0.003$). The first two RDA axes explained 30.03% and
 356 16.72% of the total variation, respectively. AK and pH showed positive correlations with the control
 357 group. In the Cd stress group, environmental factors including AK, HN, SOM, and AP were
 358 positively correlated with the samples. In the 20 μ M MT treatment group, the positively correlated
 359 factors with the samples included S-AKP, pH, and S-US; in the 50 μ M MT treatment group, S-AKP,
 360 S-US, S-CAT, and S-SC were positively correlated; and in the 100 μ M MT treatment group, SOM
 361 and AP showed positive correlations with the samples. Based on the length of the environmental
 362 vectors, AP, HN, and S-AKP were identified as the three most influential factors shaping the
 363 microbial community structure.

364 We selected the 10 bacterial ASVs from the abundance table that were most relevant to the
365 rhizosphere soil environment of daylily. Significant positive correlations were found between MT
366 treatment and *Pseudomethylobacillus*, *Lignipirellula*, and *RGDT01*. *Pseudomethylobacillus* showed
367 a negative correlation with HN and AK. Specifically, *Lignipirellula* and *RGDT01* were substantially
368 negatively correlated with SOM, AP, and HN. In contrast, *Pseudolabrys_502538*,
369 *Sphingomonas_L_486704*, *Prosthecomicrobium_A*, and *Devosia_A_502124* showed significant
370 negative correlations with MT treatment. *Pseudolabrys_502538* was significantly positively
371 correlated with SOM, AP, and HN, and negatively correlated with S-AKP. *Sphingomonas_L_486704*
372 was significantly positively correlated with SOM, AP, and AK. *Prosthecomicrobium_A* was
373 significantly positively correlated with SOM, HN, and AK. *Devosia_A_502124* was significantly
374 positively correlated with SOM, AP, and HN (Figure 10B). Mantel test results showed that plant
375 growth indicators were significantly positively correlated with S-AKP, S-SC, S-CAT, S-US, and
376 Shannon index. The plant Cd content was closely correlated with SOM, HN, AK and S-US (Figure
377 10C). Pearson correlation analysis revealed that pH was significantly negatively correlated with S-SC.
378 SOM was significantly positively correlated with HN, AP, AK and S-US, and negatively correlated
379 with Chao1 and faith's phylogenetic diversity indices. S-AKP was positively associated with S-US,
380 Shannon and Chao1 indices. S-SC was positively associated with S-CAT, and Shannon index.

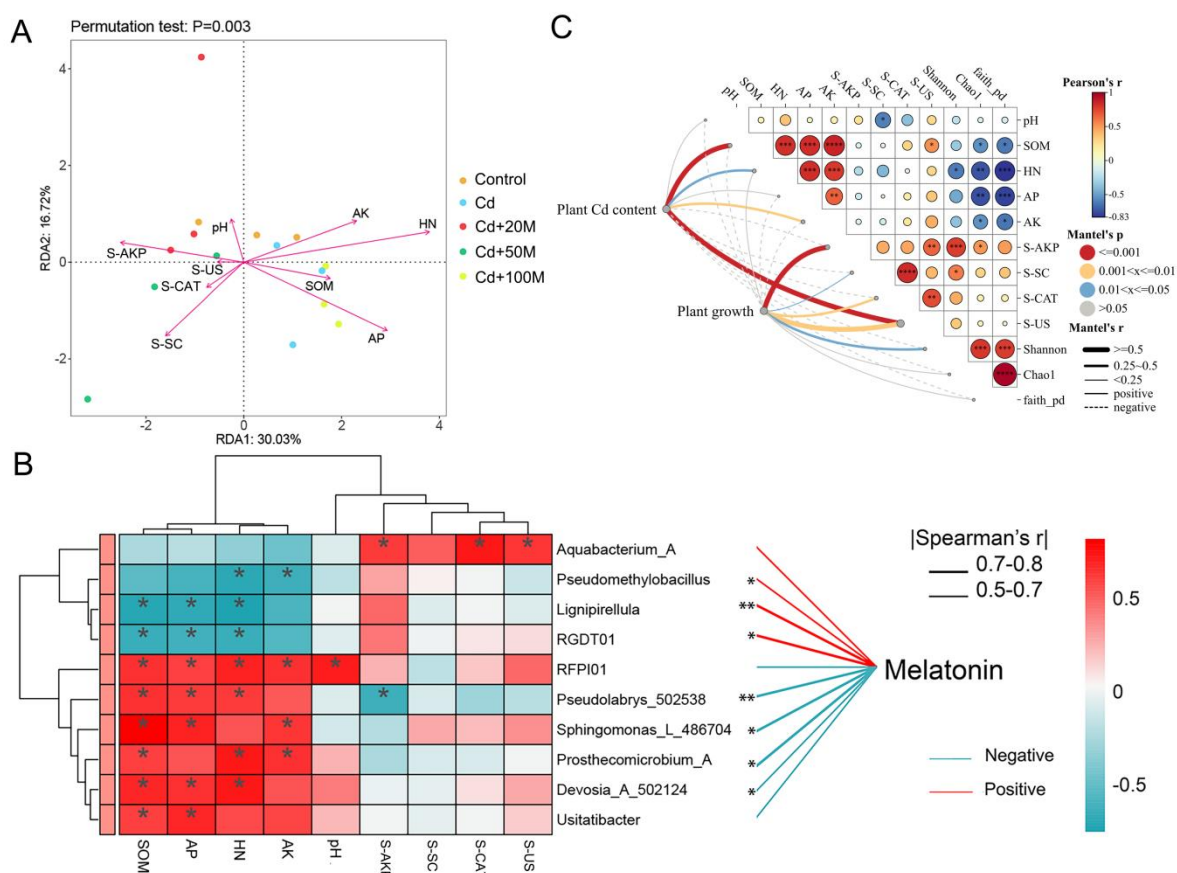


Figure 10. Correlation analysis. (A) RDA analysis. (B) Correlation analysis between MT treatment and soil environmental factors as well as bacterial communities (* $q < 0.05$, ** $q < 0.01$). (C) Mantel test analysis among soil physicochemical properties, soil enzyme activity, bacterial communities and plant growth.

PLS-PM analysis (Figure 11) showed that the variables were linked by direct and indirect pathways. The model exhibits good fit statistics ($GoF=0.65$). The coefficients of determination (R^2) for bacterial richness and plant growth were 0.56 and 0.95, respectively. Direct path analysis indicates that shoot Cd and AK content in the soil are the primary negative drivers of rhizosphere bacterial species richness. Plant growth is directly and positively influenced by bacterial abundance and S-SC activity, and negatively influenced by shoot Cd content. We also detected significant indirect effects of soil AK content and Cd content in the shoot on plant growth, mediated by bacterial abundance.

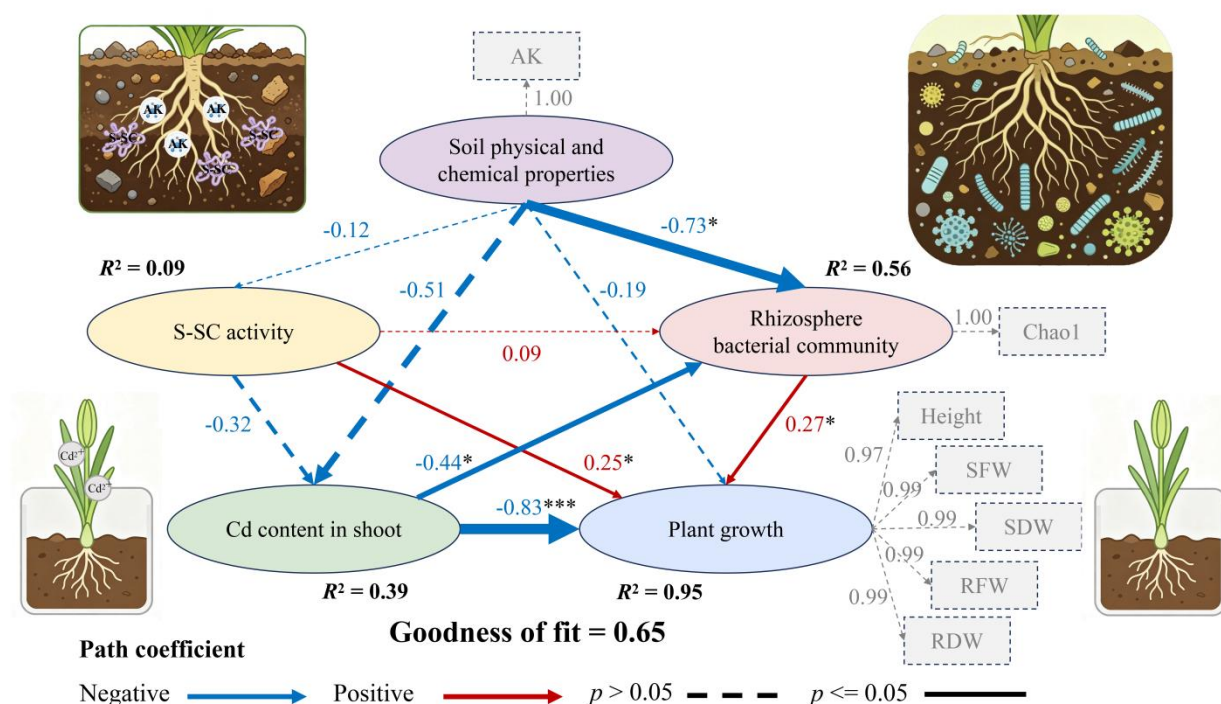


Figure 11. PLS-PM revealed direct or indirect interactions among plant growth, Cd content in shoots, soil physical and chemical properties, soil enzyme activity, and rhizosphere bacterial communities (goodness-of-fit, 0.65). Latent variables are represented by ovals, and measured variables by rectangles. R² values represent the proportion of variance explained by each variable, and line thickness corresponds to the magnitude of the path coefficients.

4. Discussion

Cd contamination in soils has become an increasingly serious global environmental issue. The use of fast-growing, high-biomass plants for remediation of heavy metal-contaminated soils represents a sustainable and environmentally friendly strategy. Daylily is a plant with remediation potential and can be applied to the phytoremediation of Cd-contaminated soils. However, when soil Cd levels exceed its tolerance threshold, daylily growth is inhibited and its remediation capacity decreases significantly. Numerous studies have shown that MT can significantly enhance plant tolerance to heavy metal stress (Li et al., 2023a), thereby improving remediation efficiency. Therefore, this study systematically investigates the mechanisms by which MT enhances daylily tolerance under Cd stress, focusing on plant growth and soil environment.

410 4.1 Effects of MT on growth and physiological indicators of daylily under Cd stress

411 As a widely distributed plant molecule, MT is involved in regulating plant adaptation to abiotic
412 stresses such as salinity, heat, and heavy metal stress (Arnao and Hernández-Ruiz, 2018). We found
413 that 20 μ M and 50 μ M MT treatments increased both plant height and biomass of Cd-stressed daylily
414 seedlings. Gu et al. (2017) also found that MT application reversed Cd-induced growth suppression in
415 alfalfa seedlings, increasing their fresh weight, dry weight and root length. MT enhances antioxidant
416 activity in plants, thereby decreasing oxidative damage and limiting ROS accumulation (Ni et al.,
417 2018). In a study of MT's effect on oilseed rape growth under Cd stress, MT application significantly
418 enhanced the activities of three major antioxidant enzymes and effectively regulated H₂O₂ and MDA
419 levels (Sami et al., 2020). Excess ROS can impair membrane integrity and cellular function, and ROS
420 overaccumulation has been shown to trigger severe oxidative damage and cell death in other
421 biological systems (Tian et al., 2019). A similar ROS-associated damage pattern has been reported in
422 fungi exposed to nerol, where elevated ROS and Ca²⁺ levels were accompanied by mitochondrial
423 dysfunction, cytochrome c release, and apoptotic features (Tian et al., 2018). Our results showed that
424 20 μ M and 50 μ M MT effectively alleviated oxidative stress in daylily under Cd stress. Specifically,
425 leaf MDA content decreased markedly, while the activities of SOD, POD, and CAT increased in a
426 coordinated manner. These findings are consistent with previous reports that MT alleviates heavy
427 metal toxicity in various plant species (Li et al., 2023a). Similar stress-physiology-growth coupling
428 has been reported in other biological systems. A large meta-analysis in aquaculture showed that
429 crowding-related stress reduced growth performance mainly by elevating physiological stress
430 markers and impairing digestive function, highlighting a clear cascade from stress response to growth
431 suppression (Song et al., 2026).

432 4.2 Effects of MT on soil physicochemical properties and soil enzyme activities under Cd stress

433 Monitoring soil physicochemical properties is essential for understanding soil fertility, guiding
434 soil management, and maintaining soil structure (Lei et al., 2026). Soil pH is a key factor governing

heavy metal mobility and maintaining soil structure and nutrient balance. The experimental results showed that soil pH gradually decreased with the increase of exogenous MT concentration. This phenomenon may be related to the physiological metabolism of roots regulated by MT. When exposed to Cd stress, MT stimulates plant roots to secrete organic acids, which can not only chelate Cd ions in soil to reduce their toxicity to plants, but also reduce soil pH by releasing hydrogen ions. Imran et al. (2022) reported similar results that MT reduces Cd mobilization in soil by promoting root exudation. SOM and available nutrients provide essential resources for plant growth and microbial processes (Hu et al., 2025). Soil nutrients are also a dominant factor shaping the composition of diazotrophic communities, with a stronger influence than climate variables and host traits (Hou et al., 2026). Under Cd stress, SOM, HN, AK, and AP in the rhizosphere soil were significantly lower than in the control, possibly because Cd suppressed soil microorganisms and root activity. 20 μ M and 50 μ M MT further reduced SOM, HN, AK and AP contents, while 100 μ M MT increased their contents. This difference may be closely related to the growth status of daylily regulated by MT. Under low concentration MT (20 μ M, 50 μ M) treatment, MT effectively alleviated Cd toxicity, promoted the growth of daylily roots and aboveground parts, enhanced the nutrient absorption capacity of plants, and thus consumed more nutrients in the rhizosphere soil, resulting in a further decrease in nutrient content (Liu et al., 2025). Soil microbial processes are jointly regulated by climate, initial soil properties, and their changes following environmental disturbance. A global meta-analysis showed that variations in soil heterotrophic respiration under nitrogen deposition were strongly associated with changes in microbial biomass and soil pH, and that their effects depended on site-specific soil conditions and interactions among environmental factors (Guo et al., 2026).

Soil enzyme activities are sensitive biological indicators of soil environmental changes, and their dynamic changes can directly reflect the impact of external stress on soil ecological function

(Ning et al., 2025). The experimental results showed that Cd stress significantly inhibited the activities of S-SC, S-CAT and S-US, and slightly reduced the activity of S-AKP, which is consistent with the research conclusion that heavy metal stress can damage the structure and function of soil enzymes (Iqbal et al., 2023). In our study, exogenous application of 20 μ M and 50 μ M MT can alleviate the inhibitory effect of Cd on soil enzyme activities to varying degrees, and 50 μ M MT has the best regulatory effect, which is specifically reflected in the significant increase of S-SC, S-CAT, S-US and S-AKP activities compared with the Cd stress group. The mechanism of MT regulating soil enzyme activities under Cd stress may be reflected in two aspects. First, MT can directly chelate Cd ions in soil, reduce the bioavailability of Cd, reduce the binding of Cd to soil enzymes, and thus protect the structure and function of enzymes from damage. Second, MT can promote the growth and reproduction of beneficial soil microorganisms, increase the number of enzyme-secreting microorganisms, and then increase the synthesis and secretion of soil enzymes (Iqbal et al., 2023). Among the four enzymes, S-CAT and S-SC showed the most significant response to MT treatment, with increases of 48.75% and 39.22% respectively, indicating that MT has a more obvious regulatory effect on soil oxidative stress and carbon cycling-related enzymes under Cd stress.

4.3 Effects of MT on rhizosphere soil microorganisms of daylily under Cd stress

Through systematic analyses of bacterial ASV richness, α/β diversity, phylum/genus composition, differential biomarkers, correlations with soil physicochemical factors, and functional potential, we found that 20 μ M and 50 μ M MT significantly relieved the inhibitory effect of Cd stress on the rhizosphere microbial community, whereas the high concentration (100 μ M) MT did not show a similar recovery effect and might even exert inhibitory effects. These results indicate that the regulatory effect of MT on soil microorganisms under Cd stress is concentration-dependent. Cd exerts a general negative effect on microbial diversity and abundance. In sensitive populations, this toxicity is often mediated through multiple pathways, including oxidative burst, membrane integrity

482 disruption, and impairment of key metabolic cycles, as documented in previous studies (Xu et al.,
483 2023). After adding 20 μM or 50 μM MT, all diversity indices bounced back markedly, with total and
484 unique ASVs even exceeding those of the control group. MT at an appropriate concentration may
485 have a stimulating effect on the rhizosphere microbiome.

486 In all treatments, the phyla Proteobacteria, Bacteroidetes, and Acidobacteria were the dominant
487 phyla. LEfSe analysis further identified biomarkers under each treatment: Cd stress significantly
488 enriched taxa such as the genus *Chiayiivirga* and the family Xanthomonadaceae, the latter often
489 causes plant diseases and is a key target for plant quarantine (Mhedbi-Hajri et al., 2011). Under 20
490 μM MT treatment, Ilumatobacter, RGDT01, and the Methylophilaceae family were enriched.
491 Members of the Methylophilaceae family are the primary bacterial group responsible for the
492 degradation of dimethyl sulfide in soil (Eyice et al., 2015). Wang et al. (2023) reported that the
493 Methylophilaceae family is positively correlated with organic matter content. Under treatment with
494 50 μM MT, the Acidimicrobiales and Burkholderiaceae were enriched. Species within the
495 Burkholderiaceae play a wide range of roles in promoting plant growth and protecting plant health
496 (Cillero et al., 2022). Under 100 μM MT treatment, the Rhizobiales and Rhodobacteraceae orders
497 were enriched, yet overall diversity decreased. Exogenous bioactive compounds may improve host
498 health by reshaping associated microbial communities. For example, rice bran peptide KF-8
499 alleviated age-related neuroinflammation and cognitive decline in mice by stabilizing key gut
500 microbes and suppressing TNF- α -mediated inflammation, highlighting a microbiota-dependent
501 mechanism of action (Mu et al., 2025). Although the host system differs, this finding supports the
502 idea that external compounds can partly act through microbiome remodeling.

503 The microbial co-occurrence network reflects interactions among taxa and community stability,
504 and is an important tool for studying how external factors regulate rhizosphere microecological
505 homeostasis (Du et al., 2025). Under 20 $\text{mg}\cdot\text{kg}^{-1}$ Cd stress, the daylily rhizosphere bacterial network
506 was severely disrupted. A global and regional analysis of soil salinization similarly showed that
507 bacterial Shannon diversity and richness declined sharply beyond a critical threshold, with

508 co-occurrence network complexity decreasing (Yang et al., 2026). At 20 μM and 50 μM , MT
509 effectively repaired the network damage caused by Cd, forming a more complex and stable microbial
510 interaction system. Muhammad et al. (2026) based on co-occurrence network analysis, found that MT
511 treatment significantly increased the complexity of bacterial networks in peanut roots.

512 RDA and Spearman's correlation analysis indicate that soil physicochemical properties are
513 important factors shaping bacterial community structure. In the control group, AK and pH correlated
514 positively with community composition, which aligns with earlier findings that pH shapes microbial
515 habitats by regulating nutrient and heavy metal solubility, while AK enters directly into microbial
516 metabolism as an essential nutrient (Shin, 2014). Under Cd stress, the positive correlations shifted to
517 AK, HN, SOM, and AP. This shift suggests that Cd disrupts the soil nutrient balance, thereby driving
518 microorganisms to seek out nutrient-rich microhabitats as a strategy to counteract toxicity. According
519 to vector length analysis, AP, HN, and S-AKP exerted the strongest influence on bacterial community
520 composition. A review by Xin et al. (2024) points out that regulating soil nutrients is an effective
521 technical approach for reducing the bioavailability of Cd in soil and modulating bacterial community
522 structure.

523 Under 20 $\text{mg}\cdot\text{kg}^{-1}$ Cd stress, our functional analysis revealed significant enrichment of pathways
524 linked to ureolysis, chitinolysis, animal parasitism/symbiosis, and human pathogenicity. Cd stress
525 can trigger localized acidification in the rhizosphere, and as microbes break down urea, the ammonia
526 released supplies a nitrogen source that helps sustain basal metabolism. Previous work has
527 demonstrated that microbial ureolysis promotes calcium carbonate precipitation, which in turn
528 immobilizes Cd (Yang et al., 2024b). Genes involved in chitinolysis were also enriched, providing
529 scarce carbon and nitrogen substrates under stress to support basal metabolism and ATP synthesis,
530 thereby helping bacteria maintain normal proliferation and improve survival under Cd stress (Kiruba
531 and Saeid, 2022). 20 μM MT mainly enriched functions related to dark oxidation of sulfur
532 compounds, dark thiosulfate oxidation, nitrate respiration, nitrogen respiration, and nitrate reduction,
533 indicating that 20 μM MT preferentially activates reductive processes in specific elemental cycles.

534 Under 50 μM MT treatment, the functions of fermentation, xylanolysis, nitrite respiration, and
535 photoheterotrophy were clearly enriched. Xylan is the primary component of hemicellulose in plant
536 cell walls. Moderate xylan degradation helps convert the carbon in moderately degradable
537 hemicellulose into a more readily available carbon source, thereby accelerating nutrient cycling in the
538 rhizosphere (Wu et al., 2024). Nitrite respiration reduces nitrite to nitric oxide or nitrous oxide.
539 Moderate nitrite respiration helps prevent the accumulation of nitrite in the rhizosphere. Studies have
540 shown that photoautotrophy is advantageous under carbon-limited conditions and promotes the
541 utilization of recalcitrant or low-energy carbon sources by bacteria (Piwosz et al., 2024). At
542 appropriate concentrations, exogenous MT shifts the metabolic focus of the rhizosphere microbial
543 community toward more active material cycling and energy acquisition, thereby helping plants
544 mitigate Cd toxicity. Under 100 μM MT treatment, functions such as phototrophy, methylotrophy,
545 and methanol oxidation were enriched. Because FAPROTAX provides taxon-based functional
546 inference, these results should be interpreted as putative functional shifts rather than direct evidence
547 of metabolic activity.

548 *4.4 Comprehensive regulation of MT on the growth and rhizosphere microorganisms of daylily under* 549 *Cd contamination*

550 Previous studies have found that soil microbial communities, soil properties, and plant growth
551 are closely related (Rong et al., 2025). In our study, the results of the mantel tests showed that plant
552 growth was correlated with bacterial richness, S-SC activity, and Cd content in the shoots. Both
553 bacterial richness and S-SC activity have a positive effect on plant growth, which is consistent with
554 previous reports that microbial diversity and soil enzyme activity promote crop productivity (Chen et
555 al., 2025; Yang et al., 2024a). In the context of Cd contamination, the intensity of soil carbon
556 metabolism, as characterized by S-SC activity, may have special ecological significance. A study by
557 Luo et al. (2023) Higher S-SC activity indicates more active decomposition of rhizosphere organic
558 matter and carbon turnover, providing an abundant energy substrate for plant-microbial interactions
559 (Yang et al., 2022; Zhang et al., 2023). At the same time, it may reduce the biological toxicity of

560 available Cd in the rhizosphere through complexation or immobilization, thereby mitigating the
561 inhibitory effects of Cd on plant growth. showed that sucrase activity is linearly correlated with soil
562 organic matter. PLS-PM clearly reveals the dual negative pathways through which Cd content in the
563 shoot affects plant growth. The significant negative correlation between shoot Cd and plant growth
564 directly stems from the toxic effects of this non-essential heavy metal, including oxidative damage
565 and impairment of nutrient transport. Shoot Cd exerts an indirect inhibitory effect by negatively
566 modulating bacterial richness. After uptake and accumulation in the plant, Cd alters both the
567 composition and amount of root exudates. Such alterations adversely restructure the rhizosphere
568 microbial community, leading to lower diversity and functional simplification, which in turn
569 diminishes the microbial capacity to promote plant growth and defend against stress. Song et al.
570 (2024) reported that heavy metals may affect plants by reshaping key microbial communities, which
571 is consistent with our results.

572 **5. Conclusions**

573 This study investigated the regulatory effects of exogenous MT on daylily seedlings and their
574 rhizosphere environment under Cd stress ($20 \text{ mg} \cdot \text{kg}^{-1}$), as well as the underlying mechanisms. The
575 results indicate that Cd stress inhibited the growth of daylilies, reduced chlorophyll and soluble sugar
576 content, induced cell membrane damage, suppressed antioxidant enzyme activity, altered soil
577 properties and enzyme activity, and reduced the richness, diversity, and structural stability of the
578 rhizosphere bacterial community. Different concentrations of MT exert varying regulatory effects,
579 with $50 \text{ } \mu\text{M}$ MT demonstrating the most effective alleviating effect. $50 \text{ } \mu\text{M}$ MT significantly
580 increased the biomass of daylily, as well as the activities of leaf protective enzymes and soil enzymes,
581 and also optimized community structure, and enriched functional microorganisms related to
582 fermentation, xylanolysis, nitrite respiration and photoheterotrophy. RDA analysis indicated that AP,
583 HN, and S-AKP were key factors shaping the rhizosphere bacterial community. Mantel test results
584 showed that plant growth indicators were significantly positively correlated with S-AKP, S-SC,

585 S-CAT, S-US, and the Shannon index, and that plant Cd content was closely correlated with SOM,
586 HN, AK, and S-US. PLS-PM indicated that shoot Cd content and soil AK were associated with
587 bacterial richness, while bacterial richness, S-SC activity, and shoot Cd content were associated with
588 plant growth. In conclusion, 50 μ M exogenous MT can alleviate Cd stress in daylilies by regulating
589 plant antioxidant defense systems, reducing the migration of Cd from roots to aboveground parts,
590 improving enzyme activities, and optimizing rhizosphere microbial community structure, providing a
591 theoretical basis for the application of MT in Cd-contaminated soil remediation and daylily
592 cultivation.

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