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# Melatonin reprograms soil microbial community, creates friendly soil environments, and promotes peanut growth

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ABSTRACT

Melatonin helps to regulate various physiological processes in plants, including growth, seed germination, and stress responses. However, the mechanism of how melatonin treatments affect soil microbe diversity and ecology, and plant growth needs to be better understood. Here, we report that melatonin coordinates interactions between soil microorganisms and root exudates to create a friendly soil environment for peanut growth under a controlled environment. Interestingly, the results showed that melatonin was capable of regulating the structure of the soil microbial community, improving its relative abundance of beneficial microorganisms (such as *Sphingomonas*, *Trichoderma*, and *Penicillium*) in the soil. Furthermore, melatonin could also change the composition of soil metabolites and nutrients. These altered soil profiles reflected a healthy environment for peanuts created by melatonin. Furthermore, the favorable growing environment increased photosynthetic performance, biomass, and peanut yield. Collectively, our findings will help us better understand the role of melatonin as a bioregulator in maintaining a healthy plant growth environment.

*Synopsis:* Melatonin treatments improved soil microbe biodiversity and enhanced plant growth and development and sustainable agricultural development.

## 1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine molecule that functions as a master regulator for plants in the form of a phytomelatonin that is essential for their growth (Arnao and Hernández-Ruiz, 2019). A variety of functions for endogenous and exogenous phytomelatonin have been reported, such as the regulation of plant growth, seed germination, cell respiration, photosynthesis, and osmolality (Reiter et al., 2014; Sharma et al., 2020). Exogenous application of melatonin has been performed via foliar sprays (Zhang et al., 2017; Zhang et al., 2017b), soil treatments (Cui et al., 2017), or seed coatings (Wei et al., 2015). In maize seedlings, melatonin applied to roots may be absorbed by the plants and then accumulated in the folia, which is the area most responsible for the photosynthesis of the plants (Yoon et al., 2019). Zhang et al. (2019) showed that low melatonin levels could regulate light signals to regulate apple flowering. Similarly, cucumbers treated with melatonin under nitrogen deficiency show increased lateral root development (Zhang et al., 2013), thus promoting the assimilation and absorption of nitrogen in cucumber plants (Qiao et al., 2019). A recent study also shows melatonin treatment improved plant tolerance to drought stress (De Camargo Santos et al., 2024).

Plant-microbe interactions, especially between roots and soil microbes, are pivotal to the evolution and contemporary ecology (Ishaq, 2017; Khan et al., 2020; Shi et al., 2024; Xue et al., 2023). Microbes and plants interact in natural environments, which may influence the composition of the rhizosphere and even bulk soil and promote the

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growth of microorganisms, improving plant fitness (Barea et al., 2002; Gouda et al., 2018). The root system secretes exudates from the apoplast to the rhizoplane, the rhizosphere, and the bulk soil by diffusion, which plays an essential role in regulating the rhizosphere of plants and the bulk soil microenvironment. Root exudates provide nutrients vital for plant growth and influence the formation of soil microbial communities (Backer et al., 2018; Eichmann et al., 2021). In plant roots, small molecules like acyl-homoserine lactones, flavonoids, nonproteinogenic amino acids, polymers, antimicrobials, or salicylic acid attract beneficial microorganisms and influence rhizosphere microbiomes, which increase plant adaptability (Bulgarelli et al., 2013). The secondary metabolites produced by these microorganisms colonizing the roots of plants, in turn, provided the plants with more adequate nutrients to promote root development (Chen et al., 2017; Chiu et al., 2022) and improved plant disease resistance (Tripathi et al., 2022; Liu et al., 2023). A study by Madigan et al. (2019) showed that melatonin added exogenously to agricultural soils without planting significantly changed the bacterial and fungal community composition (Madigan et al., 2019). Due to the importance of bacteria and fungi in soil health and crop productivity, such changes to soil microbial communities have important implications for agricultural settings (Harman et al., 2020; Mahapatra et al., 2022; Odelade and Babalola, 2019). A study reported that seed inoculated (Bio-primed) with *Paenibacillus* (T22) and *Bacillus subtilis* strains showed different accumulations of compounds, including organic acids, lipids, phenylpropanoids, organo-heterocyclic compounds, and benzenoids (Mashabela et al., 2022). Several of these compounds have been reported to be involved in interactions between plants and microorganisms, chemotaxis, biocontrol, and enhancing plant growth and development (Mashabela et al., 2022).

In plants, hormones play a critical role during the microbiome assembly, and it is not uncommon for plants and microbes to use the same hormones for different purposes(Eichmann et al., 2021). Biosynthesis of Melatonin and IAA (Indole acetic acid) synthesis are both regulated by tryptophan. Previous studies showed that microorganisms produced IAA to increase root growth (Liu et al., 2013) and suppressed pathogenic diseases (Khare and Arora, 2010; Radhakrishnan et al., 2013). Additionally, some studies have demonstrated that SA (salicylic acid) modulates specific bacteria colonization of root microbiome (Lebeis et al., 2015). As a result of these beneficial microorganisms and SA, plants can not only cope with the stress induced by external environments, but also enhance their chlorophyll content, chlorophyll fluorescence, and even the carotenoid content of their roots to encourage plant growth and development (Khan et al., 2018, 2020). Several studies have shown that melatonin treatment results in an increase in endogenous IAA levels by 1.4- to 2.0-fold when compared with untreated tomato (Tian et al., 2024) and *Brassica juncea* (Chen et al., 2009). Like IAA and SA, melatonin-treated plants also showed changes that affected soil microbiota (including bacteria and fungi) (Madigan et al., 2019). However, it is unclear whether melatonin can regulate the interaction between root exudates and soil microbes to provide a friendly living environment for plant growth and development in the soil.

Peanut, as the third largest oil crop next to soybean and rapeseed, also plays an essential role in ensuring edible oil security (Li et al., 2024b, 2024c). There is substantial evidence that melatonin can change the bacteria and fungi in soil even when added without growing plants. It can also improve plant growth, development, resilience, and resistance to biotic and abiotic stresses (Madigan et al., 2019). In our recent study, we found that exogenous melatonin improved peanut field productivity and quality at different nitrogen levels (Li et al., 2024a); we also found that nitrogene defiency affected peanut growth and development by regulating gene exptression (Li et al., 2021, 2023). However, it is unclear how melatonin regulated soil microbes. In this study, we hypothesized that melatonin mediates the root exudates, soil microbiome, and interactions between peanut roots and soil microbes to create a friendly soil environment for peanut development. Therefore, we investigated the soil metabolome, including root exudates and microbial metabolite, and the soil microbiome after peanut seeds dressed with melatonin under a controlled environmental chamber to reveal this presumption (Fig. 1). The present study delivers a new theoretical basis for understanding the interaction between melatonin and beneficial microorganisms to achieve sustainable plant growth development in the future.

## 2. Materials and methods

### 2.1. Treatment of peanut seeds and determination of plant growth indexes

#### 2.1.1. Treatment of peanut seeds with melatonin

Peanut cv. Yuhua 37 mature seeds were selected and soaked in 0.5 μM melatonin solution (0.28 g PVA and 0.32 g cellulose were added as adhesive per 100 mL solution) for 3 min. Adhesive treatment was used as a control. The treatments of sowing peanut seeds dressed with melatonin solution and only adhesive solution, respectively, were considered as PMT (planting with melatonin-dressing seed) and PCK (planting with no-melatonin-dressing seed). In addition, after removing the kernel, sowing peanut seed coats with melatonin and no melatonin, respectively, were treated as NPMT (no indeed planting with only melatonin-dressing seed coat) and NPCK (no indeed planting with only no-melatonin-dressing seed coat). Air-dried peanut seeds or seed coats were grown in pots ($\Phi = 17$ cm, $H = 19$ cm) with soil from the experimental base of Yanjin County, Xinxiang City, Henan Province (114°0.20′ E, 35°0.14’ N). Fifteen days after planting, urea, diammonium phosphate, and potassium sulfate, as fertilizers, were applied for all treatments according to the 67.5 kg N, 112.5 kg $P_2O_5$, and 112.5 kg $K_2O$ per hectare.

#### 2.1.2. Sampling and harvesting

After 55 days of peanut planting, the whole plants and the soil were harvested during peg-setting. First, the surface soil of 8 cm in depth was carefully removed. Second, the remaining soil in the pot and plant was slowly and carefully poured out into one large and shallow basin disinfected with alcohol, and the roots of the plant were gently shaken, allowing the soil adhering to the roots to fall into the basin. Finally, the whole plant was used to determine both fresh and dry biomass, flower number, and peg number. The sieve of 14 meshes was used to separate some broken roots and large clod. The sieved soils treated with PMT, PCK, NPMT, and NPCK were, respectively, collected in 50 mL test tubes and frozen in liquid nitrogen for soil microbe and soil metabolomics analysis. After the 100 days of peanut planting, the plant was harvested for yield and mature pod number.

#### 2.1.3. Photosynthesis, chlorophyll fluorescence, and nitrogen balance index determination

One day before the first harvest, the net photosynthetic rate (Pn) was determined using the LI-6800 (LI-COR, America); the Handy PEA plant efficiency analyzer (Hansatech, MK) was used to determine chlorophyll fluorescence characteristics of peanut leaves after the dark adaptation clip was clamped on the peanut fully opened leaves and the leaves adapted in darkness for at 30 min. The nitrogen balance index and chlorophyll content of the front and back of one leaflet per plant were measured by the Dualex scientific instrument (France).

#### 2.1.4. Determination of plant biomass of peanut, yield, and number of flowers, pegs, and pods

After the first harvest, the number of flowers, needles, and different-sized immature pods of peanut plants were first counted. Then, each plant’s stems, leaves, roots, and pods were separated and stored into calfskin bags, which were first defoliated at 105°C and then dried at 60°C. The dry weight of each part of the peanuts was weighed. After the final harvest, the mature pods were counted, and the pod’s weight per plant was weighed after air-drying.

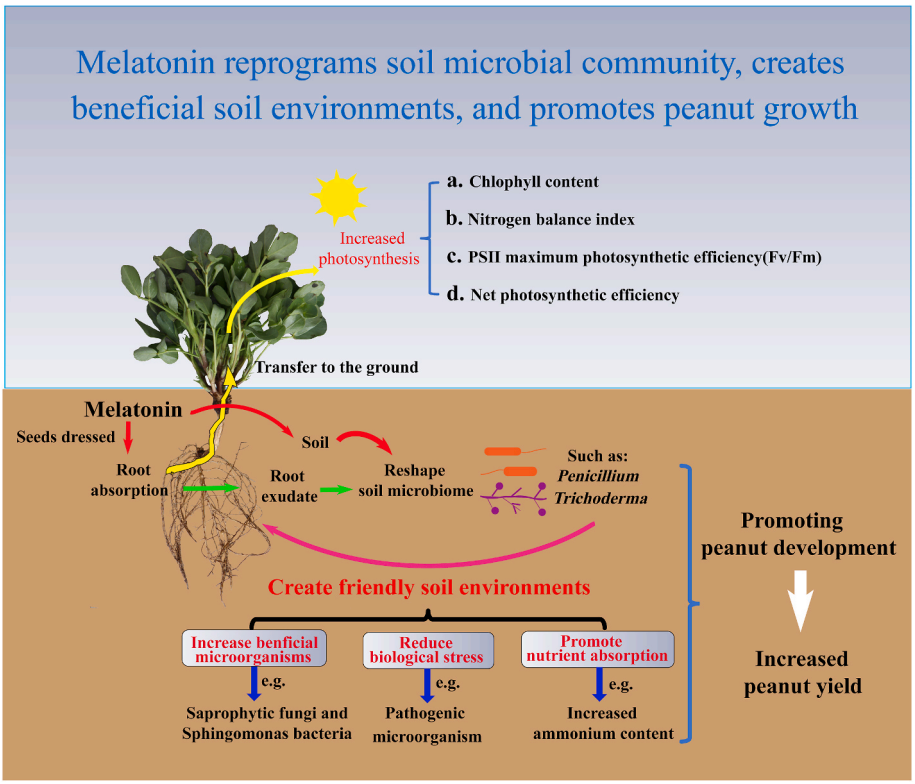


**Fig. 1.** Melatonin mediated peanut-soil microbiome-soil metabolite interaction. Melatonin regulates the components of peanut root exudates and reshapes soil microorganisms' composition; increasing the abundance of beneficial microorganisms can improve the absorption of nitrogen, phosphorus, and potassium and pathogen resistance of peanuts, and provide an excellent growing environment for the growth and development of peanut plants, to improve its yield. This study was repeated for 4–12 times.

### *2.2. Detection of physiological and biochemical indexes of soil under different melatonin treatments*

According to an indol blue method previously reported by Hood--Nowotny et al. (2010), the content of ammonium nitrogen was determined in the soil. The modified detection method of Zhao and Wang (2017) was used to detect the nitrate nitrogen content in the soil. The amount of available phosphorus in the soil has been determined using the method developed by Nagul et al. (2015). A conventional method has been used to determine the soil's pH and electrical conductivity (EC).

### *2.3. 16S rRNA and ITS analysis of soil microorganisms*

We thawed four soil samples on ice and freeze-dried them to constant weight before extracting DNA from each sample with the Power Soil DNA Isolating Kit (Mo Bio Laboratories, Inc., Carlsbad, USA). 16S rRNA) and ITS1 libraries were created by using a two-step PCR method by Illumina, where bacterial primers refer to a previous report of Klindworth et al. (2013) and fungal primers ITS1F and ITS2 refer to Gardes and Bruns (1993) and White et al. (1990), respectively. We quantified amplicons with a Qubit 2.0 fluorometer (Thermo Fisher Scientific, USA), diluted to 1 ng/L, and sequenced them with MiSeq (PE250). The raw reads for bacteria and fungi are shown in Tables 1 and 2, obtained from the 16 samples of soils. We have submitted these raw reads in the NCBI (PRJNA917116) as part of a present study. We employed BLAST$^{+}$ to identify the operational taxonomic units (OTUs) of bacteria and fungi (Camacho et al., 2009), using the 16S SILVA database (Silva 132) for bacteria and the UNITE fungal ITS database release version 7.1 for fungi (Nilsson et al., 2019). We computed microbial diversity as described previously by Grice et al. (2009).

QIIME2 was employed to analyze the Alpha and $\beta$ diversity of differently treated soil samples to reveal the differences in soil microorganisms among the samples. Non-Dimensional Scaling (NMDS) demonstrated the separation of samples treated with different melatonin. We used a heatmap to reveal the similarities and differences in community composition among samples treated with different melatonin. ANOVA analysis showed species-level differences between bacteria and fungi. Co-occurrence network analysis of soil microflora was performed using the BMK Biocloud platform software. FAPROTAX and FUNGuild were used to predict the function of significantly different bacteria and fungi, respectively, via the Biocolor platform.

**Table 1**
Identification of 16S rRNA soil bacteria data under different melatonin treatments.

| Sample ID | Raw Reads | Clean Reads | GC (%) | Q20 (%) | Q30 (%) | Effective (%) |
|---|---|---|---|---|---|---|
| NPCK1 | 80151 | 79809 | 57.48 | 99.02 | 96.09 | 97.4 |
| NPCK2 | 80044 | 79683 | 57.48 | 99.04 | 96.13 | 97.26 |
| NPCK3 | 79856 | 79502 | 57.51 | 99.04 | 96.14 | 97.46 |
| NPCK4 | 79847 | 79466 | 57.5 | 99.02 | 96.08 | 97.51 |
| NPMT1 | 79507 | 79138 | 57.65 | 99.03 | 96.11 | 97.87 |
| NPMT2 | 79817 | 79461 | 57.78 | 99.03 | 96.13 | 97.76 |
| NPMT3 | 79721 | 79379 | 57.73 | 99.05 | 96.18 | 97.79 |
| NPMT4 | 80667 | 80291 | 57.67 | 99.01 | 96.04 | 97.39 |
| PCK1 | 80061 | 79685 | 57.25 | 99.02 | 96.07 | 97.29 |
| PCK2 | 79749 | 79374 | 57.4 | 99.03 | 96.13 | 97.67 |
| PCK3 | 80039 | 79659 | 57.31 | 99.06 | 96.21 | 97.73 |
| PCK4 | 80127 | 79804 | 57.43 | 99.04 | 96.13 | 98.11 |
| PMT1 | 80376 | 80023 | 57.32 | 99.04 | 96.16 | 97.27 |
| PMT2 | 80200 | 79857 | 57.34 | 99.05 | 96.17 | 97.54 |
| PMT3 | 80062 | 79725 | 57.39 | 99.06 | 96.2 | 97.12 |
| PMT4 | 79902 | 79546 | 57.39 | 99.02 | 96.08 | 97.08 |

**Table 2**
Identification of ITS soil fungi data under different melatonin treatments.

| Sample ID | Raw Reads | Clean Reads | GC (%) | Q20 (%) | Q30 (%) | Effective (%) |
|---|---|---|---|---|---|---|
| NPCK1 | 79750 | 79361 | 48.13 | 99.74 | 98.71 | 97.41 |
| NPCK2 | 80205 | 79861 | 48.27 | 99.73 | 98.64 | 98.20 |
| NPCK3 | 79936 | 79562 | 48.18 | 99.73 | 98.65 | 97.88 |
| NPCK4 | 78363 | 78030 | 48.35 | 99.76 | 98.78 | 98.10 |
| NPMT1 | 79793 | 79409 | 45.99 | 99.56 | 98.00 | 97.21 |
| NPMT2 | 80099 | 79764 | 47.25 | 99.67 | 98.42 | 97.81 |
| NPMT3 | 80142 | 79825 | 48.17 | 99.75 | 98.72 | 97.59 |
| NPMT4 | 79821 | 79458 | 47.83 | 99.75 | 98.72 | 98.14 |
| PCK1 | 80051 | 79289 | 49.51 | 99.25 | 97.12 | 96.53 |
| PCK2 | 77858 | 77422 | 49.04 | 99.64 | 98.42 | 98.09 |
| PCK3 | 55942 | 55654 | 48.71 | 99.75 | 98.77 | 98.22 |
| PCK4 | 50957 | 50682 | 48.53 | 99.68 | 98.55 | 98.05 |
| PMT1 | 80033 | 79645 | 47.19 | 99.76 | 98.76 | 98.36 |
| PMT2 | 71961 | 71603 | 46.72 | 99.76 | 98.79 | 98.11 |
| PMT3 | 75902 | 75486 | 47.84 | 99.68 | 98.53 | 97.52 |
| PMT4 | 74243 | 73843 | 48.04 | 99.72 | 98.67 | 98.01 |

### 2.4. Non-targeted metabolic profiling analysis of the effect of melatonin on peanut-mediated soil metabolites

#### 2.4.1. UPLC-MS/MS analysis

To examine the impact of melatonin treatment on the metabolites of microorganisms in the soil and the root exudates of peanut plants, a non-targeted soil metabolomics analysis was conducted using an ACQUITY UPLC I-Class plus (Waters Corporation, Milford, USA) in conjunction with a Q-Exactive quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm) was employed to analyze metabolite profile under both positive and negative ESI ion modes. The following solvent system was employed: mobile phases A (0.1% formic acid) and mobile phases B [Acetonitrile/Methanol 2/3(v/v) +0.1% formic acid]. Gradient program: 1% B at 0 min, 30% B at 1 min, 60% B at 2.5 min, 90% B at 6.5 min, 100% B at 8.5 min, 100% B at 10.7 min, 1% B at 10.8 min, and 1%B at 13 min. The flow velocity and column temperature were maintained at 0.35 mL/min and 45°C, respectively. The injection volume of the samples was two μL. The full scan mode was employed to acquire data, with a range of 50 m/z to 1000 m/z, and was combined with the MSE mode. Furthermore, distinct collision energies (CE) acquired two distinct scans intermittently during the run. Mass spectrometry parameters consist of a low-energy (CE 4 eV) scan and a high-energy (CE gradient 20–45 eV) scan. These scans were employed to disperse the ions using argon (99.999%), a collision-induced dissociation gas. The mass spectrometry program was executed with a scan time of 0.2 s, an interscan delay of 0.02 s, a cone voltage of 40 V, a capillary voltage of 2.5 kV, a source temperature of 115°C, a desolvation gas temperature of 450°C, and a desolvation gas rate of 900 L/h.

#### 2.4.2. GC-MS/MS analysis

In order to identify additional metabolites in the above soil samples, they were subjected to further analysis using the Agilent 7890B gas chromatography system in conjunction with the Agilent 5977A MSD system (Agilent Technologies Inc., CA, USA). The derivatives of soil metabolites were separated using a capillary column (length: 30 m; inner diameter: 0.25 mm; membrane thickness: 0.25 μm, Agilent J & W Scientific, Folsom, CA, USA). Helium was adopted as the carrier gas, with a constant flow rate 1 mL/min in the column. The injector temperature was maintained at 260°C. The splitless mode was employed to administer a one μL injection volume. The temperature program started with an initial oven temperature of 60°C, which was maintained for 0.5 min. Subsequently, the temperature was increased to 125°C and then 210°C at 8°C per min, followed by 270°C at 15°C per min. The final stage elevated the temperature to 305°C over 5 min at 20°C per minute. The mass spectrum conditions include an electron-bombarded ion source (EI), a quadrupole temperature of 150°C, an ion source temperature of 230°C, and an electron energy of 70 eV. Full scan mode (SCAN), quality scan range: 50–500 m/z.

### 2.5. Correlation analysis of soil microbiome and metabolomic data

We analyzed the association of 16S rRNA and ITS with non-target metabolomics samples, respectively, and used MetOrigin analysis software (http://metorigin.met-bioinformatics.cn/home/) to explore the correlation of soil microorganisms with metabolites.

### 2.6. Statistical analysis

The photosynthesis parameters, chlorophyll fluorescence parameters, nitrogen balance index, peanut growth, and development parameters, yield pod number, soil properties, soil metabolomics, and soi microbiome were measured using biological replicates ranging from 4 to 12. Data analysis was conducted with GraphPad Prism 9.0 ($P < 0.05$). The differential metabolites were identified at the projection (VIP) and FC of $\leq$0.83 or FC $>$ 1.2. The MetaboAnalystrR R libraries were employed to conduct orthogonal partial least squares-discriminant analysis (OPLS)-DA. The differential metabolites were subjected to functional enrichment analysis using the KEGG database (http://www.genome.jp/KEGG/pathway.html). The hypergeometric test was employed to determine the significance P value threshold of the KEGG enrichment analysis, which was subsequently corrected for multiple testing using the Benjamini-Hochberg method. Permutational multivariate analysis of variance (PERMANOVA) was implemented to evaluate the data obtained from various experiments that demonstrated statistically significant differences at an *p*-value of less than 0.05.

## 3. Results

### 3.1. Effects of melatonin treatment on photosynthesis in peanut

As an important factor in improving plant growth, the photosynthetic efficiency of peanut was measured at the peg setting stage of the early flower season under different melatonin treatment conditions. According to the results, melatonin treatment significantly enhanced peanut chlorophyll content (Fig. 2b) and NBI nitrogen balance index (Fig. 2c); The maximum photosynthetic efficiency (Fig. 2d) and net photosynthetic efficiency (Fig. 2e) of peanut were significantly increased for melatonin treatment. The results of this study indicated that the peanut N nutrition index, as well as the efficiency of photosynthetic processing, can be significantly improved by melatonin treatment.

### 3.2. Effects of melatonin on peanut biomass and pod yield per plant

The photosynthesis increase reflected good plant growth and development, so the change in peanut plant biomass was analyzed. Melatonin (0.5 μM) treatment significantly increased the number of needles (Fig. 3a), immature pods (Fig. 3b), stem and leaf dry weight (Fig. 3d), root dry weight (Fig. 3e), and total immature pod dry weight per plant (Fig. 3f) in peanuts as compared to the control. However, melatonin treatment has no effect on the number of flowers (Fig. 3c). These results indicated that melatonin could promote the growth and development of peanuts.

The growth-promoting effect of melatonin on peanut plants was also reflected in the final pod yield and mature pod number per plant. The results demonstrated that melatonin could significantly increase dry weight and number of pods by 28.7% and 22.08%, respectively (Fig. 4a and b). These results indicated that melatonin had an important application value in increasing peanut yield.

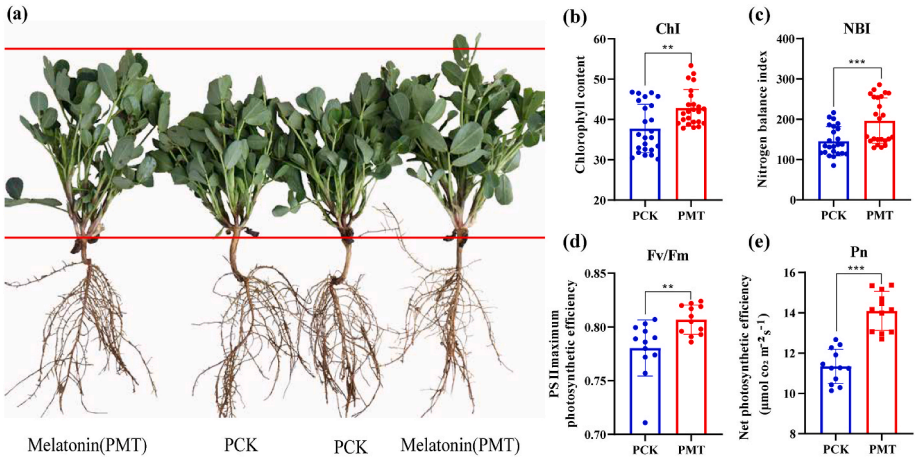


**Fig. 2.** Effects of melatonin treatment on peanut photosynthesis during peg setting period. (a) Melatonin regulates the growth phenotype of peanuts under melatonin coating treatment (PMT). (b) Chlorophyll content, $^{*}P < 0.05$. (c)Nitrogen balance index (NBI), $^{***}P < 0.001$. (d) PSII maximum photosynthetic efficiency (Fv/Fm), $^{**}P < 0.01$. (E)Net photosynthetic efficiency, $^{***}P < 0.001$. This study was repeated for 12 times.

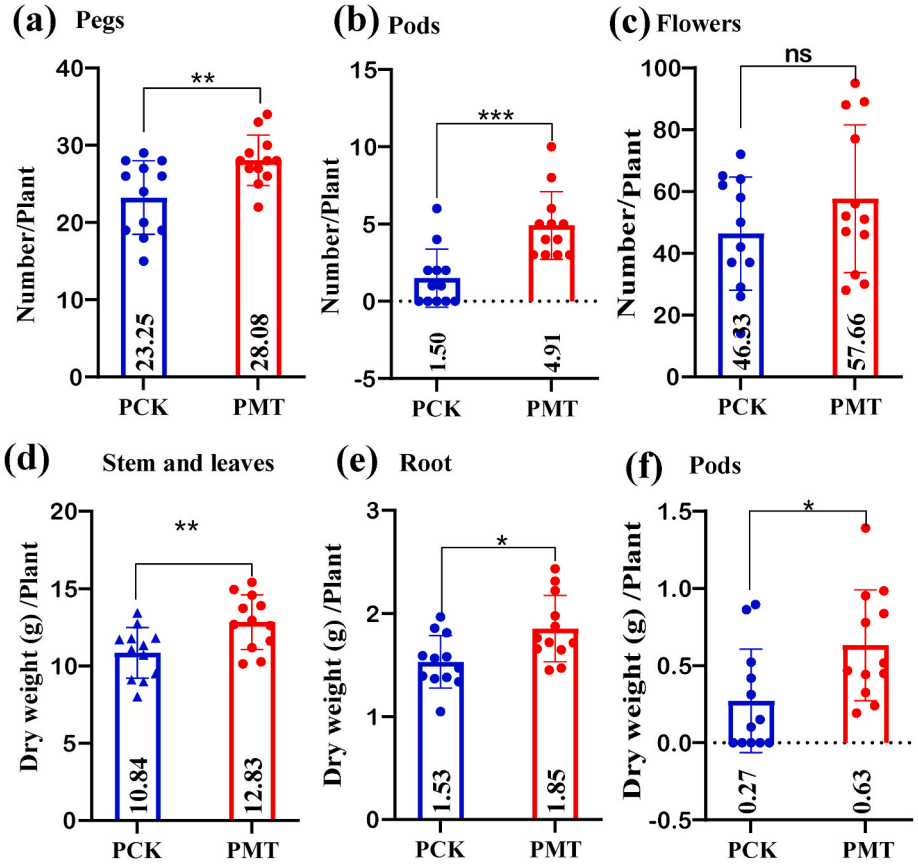


**Fig. 3.** Effects of melatonin treatment on peanut biomass and number of reproductive organs during peg setting period. (a) Number of needles, $^{**}P < 0.01$. (b) Number of pods, $^{***}P < 0.001$. (c) Number of flowers, ns: No significant difference. (d) Stem and leaves dry weight, $^{**}P < 0.01$. (e) Root dry weight, $^{*}P < 0.05$. (f) Pods dry weight, $^{*}P < 0.05$. This study was repeated for 6 times.

### *3.3. Detection of physiological and biochemical indexes of soil under different melatonin treatments*

The soil's physiological and biochemical indexes were analyzed to explore further how melatonin regulates the soil environment to promote peanut growth and development. The results showed that the contents of ammonium nitrogen ($P = 0.0003$), nitrate nitrogen ($P = 0.1736$), and available phosphorus ($P = 0.0903$) were increased after PMT treatment compared with PCK treatment (Fig. 5a, b, c). In addition, the EC and pH of soil treated with melatonin (PMT) were decreased compared with PCK (Fig. 5d and e). To clarify the effect of melatonin on soil physicochemical properties, it was found that melatonin could increase the content of ammonium nitrogen and available phosphorus by comparing NPMT and NPCK(Fig. 5a–c). However, the nitrate nitrogen ($P = 0.0001$) and available phosphorus ($P = 0.0979$) content were decreased, and pH value ($P = 0.0001$) and ammonium nitrogen ($P =$

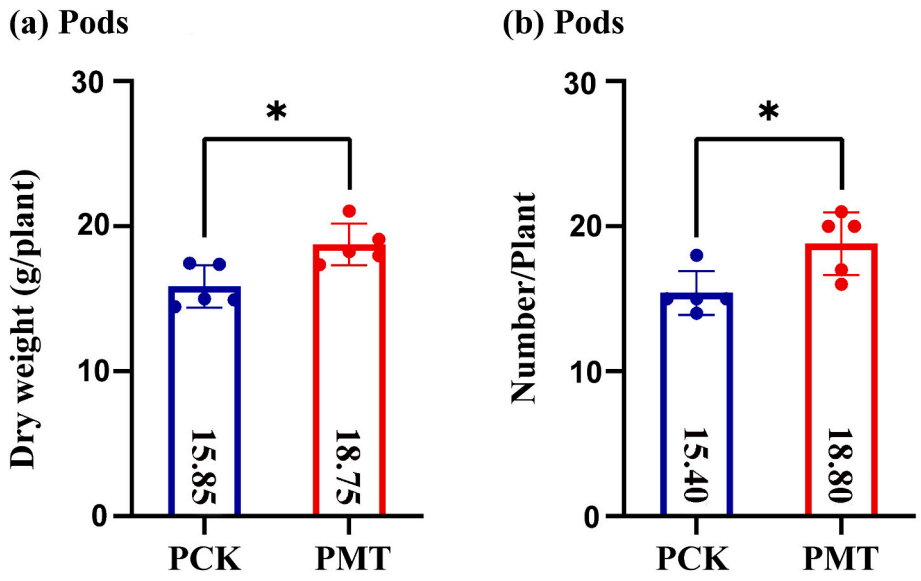


**Fig. 4.** Effects of melatonin seed dressing on peanut yield and mature pod number at pod maturity end. (a) The effect of melatonin on pod dry weight. (b) The effect of melatonin on pod numbers. *$P < 0.05$. This study was repeated for 6 times.

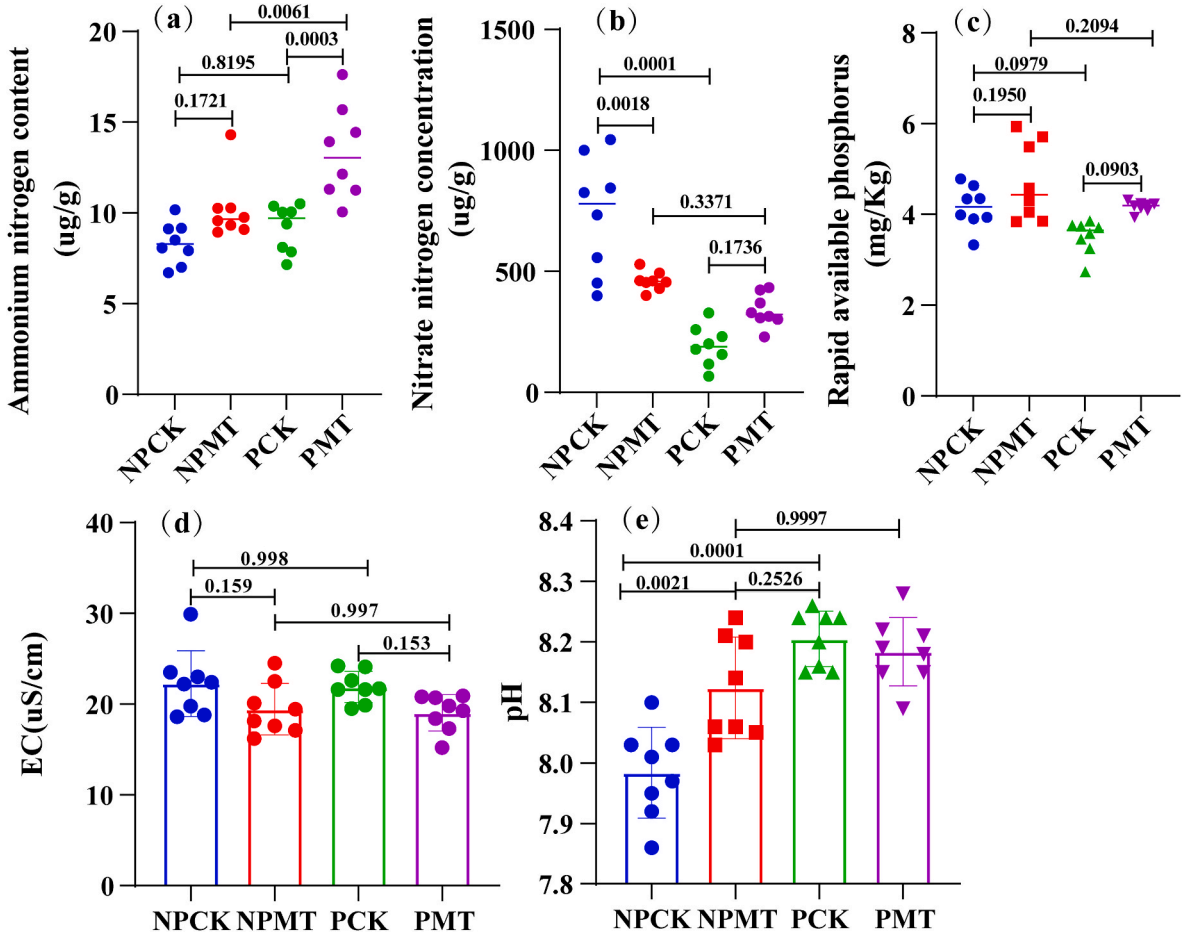


**Fig. 5.** Physiological and biochemical indexes of soil under different melatonin treatments. (a) Ammonium nitrogen content. (b) Nitrate nitrogen concentration. (c) Rapidly available phosphorus. (d) Conductivity. (e) Soil pH in peanut-planted soil. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. This study was repeated for 6 times.

0.8195) content was increased under PCK treatment compared with NPCK (Fig. 5). In summary, these results potentially proofed that melatonin treatment increased nitrogen and phosphorus content in the soil to promote peanut growth, and decreased soil EC to promote ion absorption, to maintain an excellent growing environment for peanuts.

### 3.4. Melatonin affects soil microbial diversity and abundance

To explore the function of melatonin in regulating the peanut soil microenvironment, soil microbial diversity was analyzed. α diversity results showed that PMT had a certain degree of influence on soil bacterial and fungal diversity compared with PCK (Figs. S1a and d). In addition, comparing NPMT and NPCK, it was found that melatonin itself had a more significant effect on the α diversity of soil microorganisms (Figs. S1a and d). These results proved that melatonin affects soil microbial community structure in conditions of with and without plant. PERMANOVA revealed significant variation in bacterial and fungal β diversity in PMT compared with PCK or NPCK (Figs. S1b and e). Non-metric Position scaling (nMDS) ordination also showed the clear separation responses of microbial communities to different melatonin treatments (Figs. S1c and f). These findings revealed that melatonin-mediated peanut plants had significant effects on soil microbiota.

### 3.5. Response of soil microbiota to melatonin

The heatmap showed changes in bacterial and fungal abundance between different melatonin treatments. Compared with PCK, relative abundance of PMT was significantly increased, including 7 bacteria: *Nitrospira*, unclassified_*Geminicoccaceae*, unclassified_*Anaerolineaceae*, uncultured_soil_bacterium, *RB41*, Unclassified_*Chloroflexi*, *Bryobacter* (Fig. 6a) and 7 fungi: *Humicolopsis*, *Solicoccozyma*, *Trichoderma*,unclassified_*Leotiomycetes*, *Humicola*, *Saitozyma*, *Aspergillus* (Fig. 6b). The relative abundance was significantly reduced, which includes two bacteria: *Subgroup*_10, unclassified_*Chitinophagaceae* (Fig. 6a) and three fungi: *Talaromyces*, *Trichocladium*, *Entoloma* (Fig. 6b). These results indicated that melatonin had a regulatory effect on specific soil microbial flora. Additionally, we compared soil microbiota between PMT and NPCK. We found that the relative abundance of 12 strains of bacteria and 11 strains of fungi significantly increased, while 15 strains of bacteria and eight strains of fungi decreased significantly (Fig. 6a and b). In addition, in comparison of NPMT and NPCK, the relative abundance of 12 bacterial genera and four fungal genera was significantly increased. In contrast, the relative abundance of 4 bacterial and three fungal strains significantly decreased (Fig. 6a and b). These results indicated that the treatment of plants with melatonin had a regulatory effect on specific soil microbial flora.

One-way ANOVA was used to reveal further the effect of melatonin treatment on specific soil microbial strains. The results showed that the relative abundance of *Flacisolibacter* bacteria and *Calonectria* fungi was significantly increased in PMT treatment compared with PCK treatment (Fig. 6c and d). Compared with NPCK, PMT included three bacteria (uncultured_ *Actinomycetales*_bacterium, *Sphingomonas*, *Flacisolibacter*) and four fungi (unclassified_ *Glomeraceae*, *Trichocladium*, *Rhizoctonia*, *Entoloma*) relative abundance increased significantly, and the three

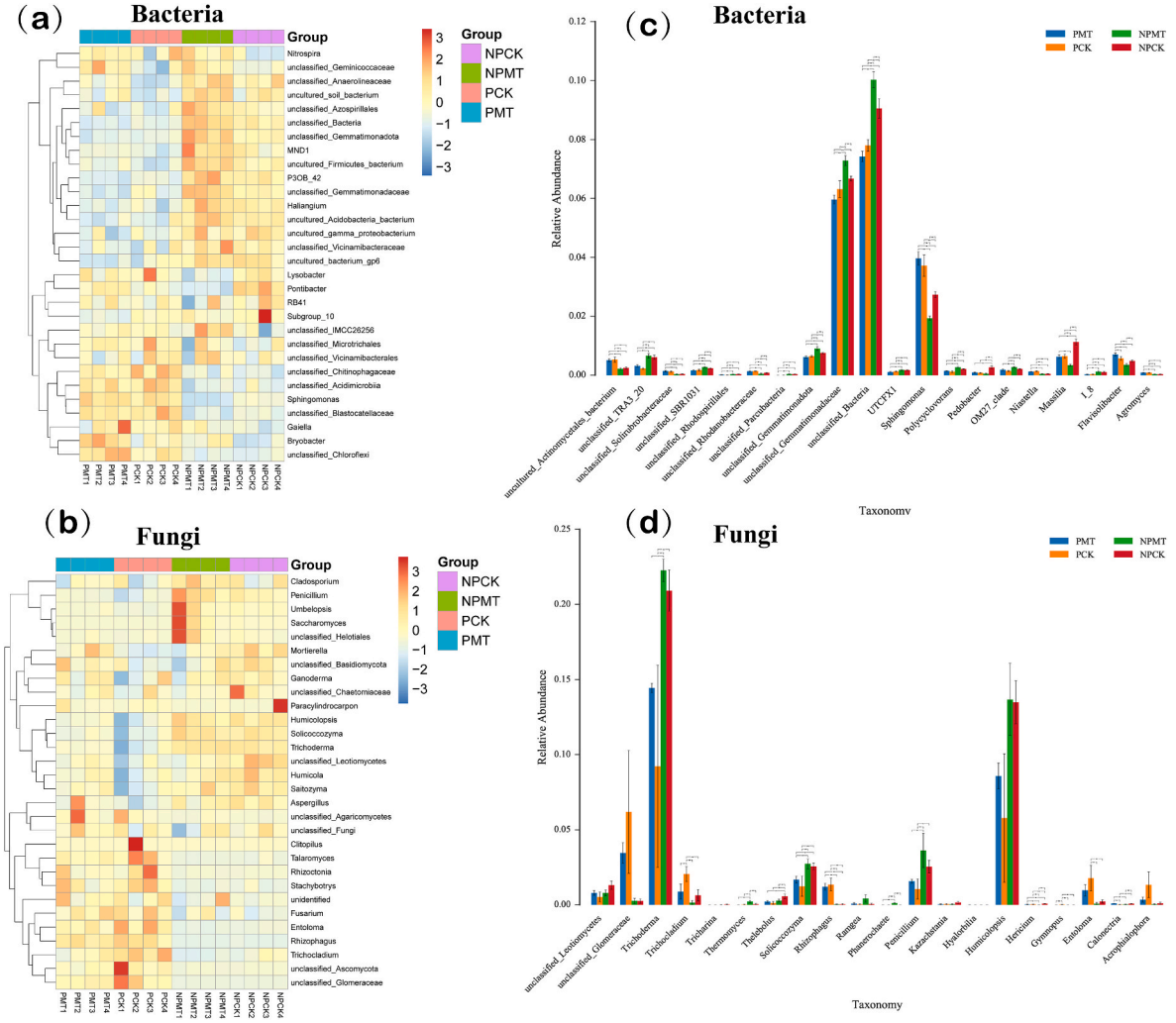


**Fig. 6.** Response of soil microbiota to melatonin. (a, b) PMT, PCK, NPMT, and NPCK treated peanut plant differential soil bacterial and fungal relative abundance cluster heat map. (c, d) Univariate variance analysis of the relative abundance of soil bacteria and fungi in different peanut plants treated by PMT, PCK, NPMT, and NPCK. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

fungi (*Solicoccozyma*, *Penicillium*, *Humicolopsis*) significantly were decreased in relative abundance (Fig. 6c and d). These results further demonstrate the potential function of melatonin in regulating soil microbial community structure, which is conducive to peanut growth and development.

### 3.6. Co-occurrence network analysis of microflora in the soil

The Co-occurrence network analysis of microflora in the soil showed that *Trichoderma*, *Penicillium*, *Solicoccozyma*, *Ganoderma*, *Talaromyces,* and unclassified *_Ascomycota* became the dominant strains under PMT treatment compared with PCK treatment (Fig. 7a). Among them, *Trichoderma* ($P = 0.0123$) and *Penicillium* ($P = 0.0195$), as the main bacterial groups with the ability to promote plant growth and regulate soil microorganisms, the relative abundance of the two genera increased significantly under PMT treatment compared with PCK (Fig. 7b). These results further proved that melatonin affected the soil microflora structure and changed the dominant microflora.

### 3.7. Functional prediction of soil microbiota in response to melatonin

FAPROTAX bacterial function prediction and FUNGuild fungal function prediction were performed to further analyze the function of soil microorganisms under melatonin treatment. The current findings showed that PMT increased the relative abundance of bacteria in aerobic_chemoheterotrophy, chemoheterotrophy (Fig. 8a), and fungi in saprotroph (Fig. 8b). In contrast, the relative abundance of bacteria in fermentation and fungi symbiotroph and pathotroph were decreased compared with PCK treatment (Fig. 8a and b). However, compared with NPCK, PMT increased the relative abundance of bacteria in chemoheterotrophy, aerobic_ chemoheterotrophy, nitrate_reduction and fungal symbiotroph function, while in bacterial chitinolysis, predatory_or_exoparasitic, ureolysis and the fungus saorotroph, pathotroph are functionally reduced in relative abundance (Fig. 8a and b). These results indicated that melatonin could regulate specific bacterial and fungal populations to maintain a certain homeostatic environment of peanut plants, which was conducive to autogenous growth and development.

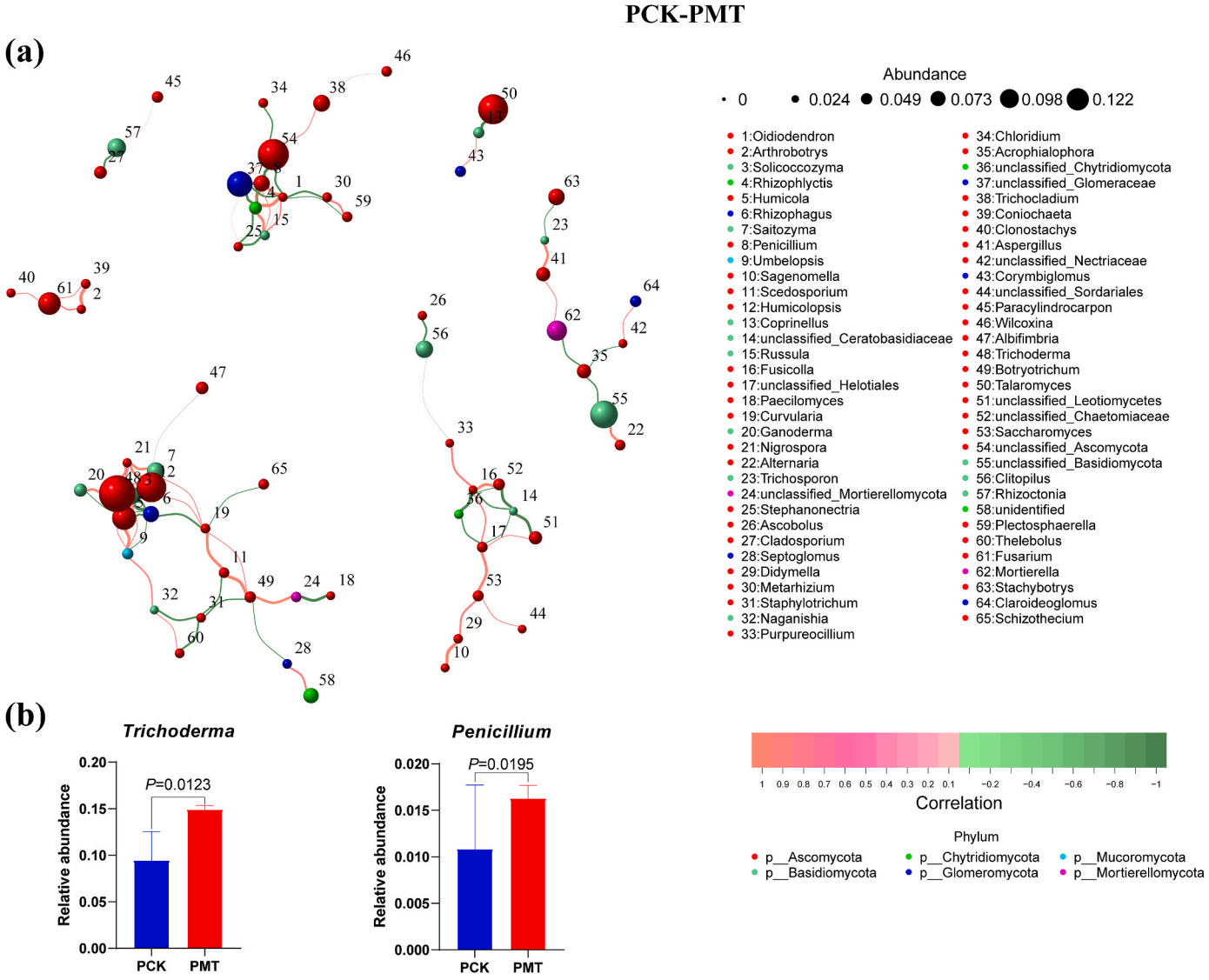


**Fig. 7.** Co-occurrence network analysis of soil microflora in the soil. (a) Co-occurrence network analysis for the bacteria and fungus under different melatonin treatments. Node size represents the relative abundance of strains in different genera, and the node color represents the strain classification on the phylum. (b) Relative abundance expression of *Trichoderma* and *Penicillium* strains. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

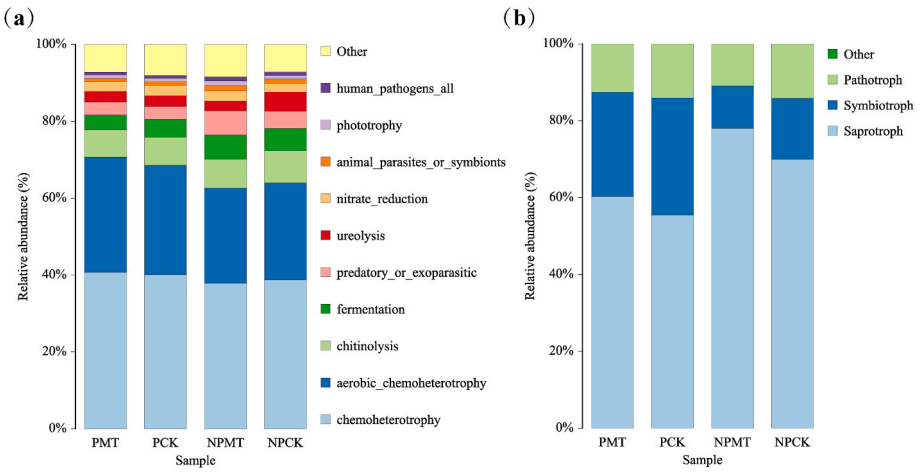


**Fig. 8.** Functional prediction of soil microbiota in response to melatonin. (a) FAPROTAX bacterial function prediction. (b) FUNGuild fungal function prediction.

### *3.8. Soil metabolomics analysis associated with melatonin-treated peanut plants*

Untargeted metabolomics analysis was used to reveal how melatonin regulates soil metabolites to maintain peanut function in a favorable growing environment. The OPLS-DA analysis showed a separation between PMT and PCK, indicating that VIP could be used to identify differential metabolites (Figs. S2a and b). We identified 151 differential metabolites, 57 of which showed upregulated and 94 of showed downregulated (Fig. 9). These metabolites are grouped into 11 broad categories, including that 18 DEMs were classified into lipids (11.92%), 13 DEMs were classified into fatty acid (13.9%), 11 DEMs were classified into amino acid and its derivative (7.28%), 24 DEMs were classified into alkaloid (15.89%), 22 DEMs were classified into phenolics (14.57%), 11 DEMs were classified into carbohydrates (7.28%), 6 DEMs were classified into terpene (3.97%), 17 DEMs were classified into Other (11.26%),

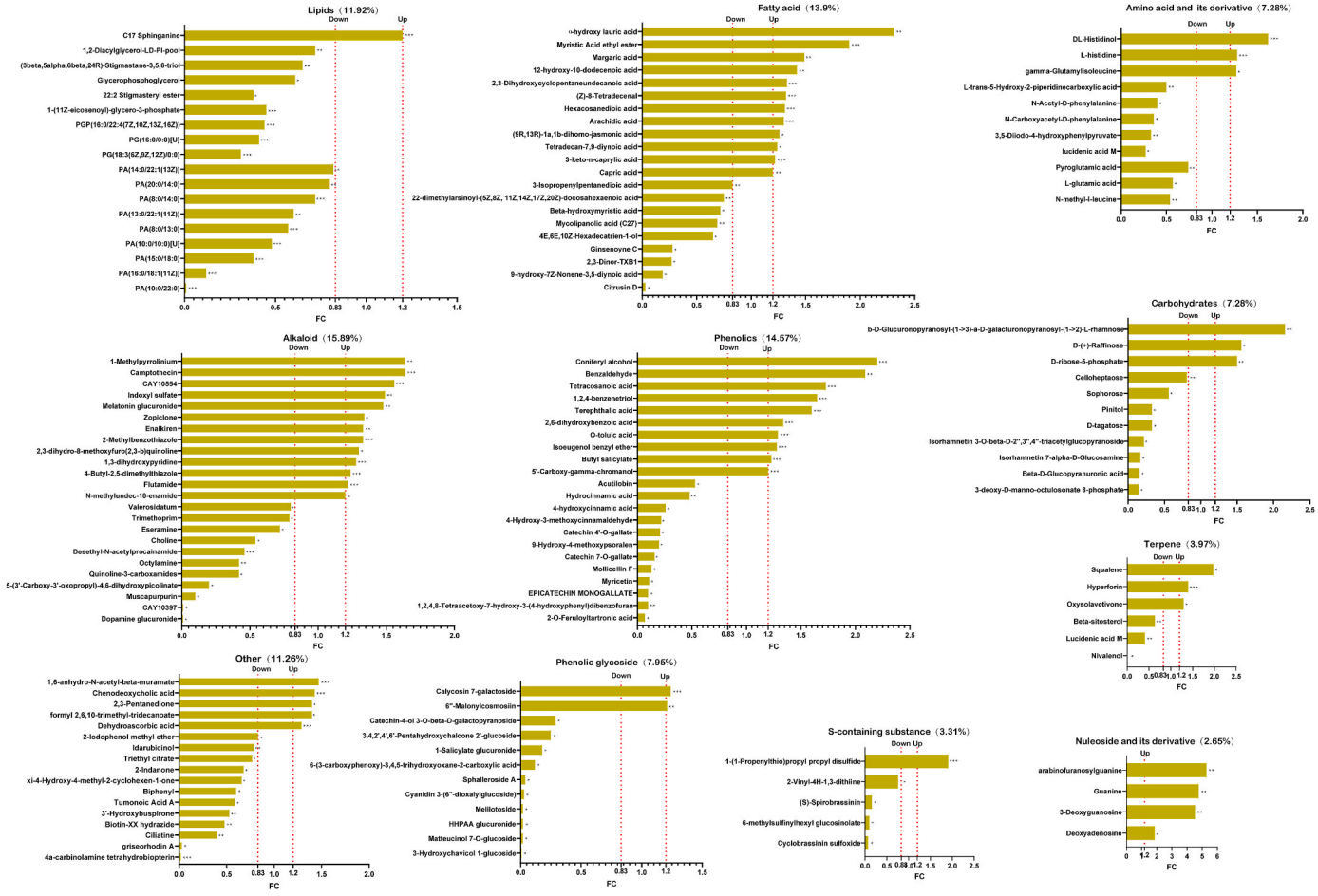


**Fig. 9.** Soil metabolomics analysis under melatonin stress peanut plants. Significant differences in the classification and proportion of soil metabolites, and up-regulated (FC $\geq$ 1.2) and down-regulated (FC $\leq$ 0.83) metabolites. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. This study was repeated for 6 times.

12 DEMs were classified into phenolic glycoside (7.95%), 5 DEMs were classified into S-containing substance (3.31%), and four DEMs were classified into nucleoside and its derivative (2.65%) (Fig. 9). Among these metabolites, we found that the content of a lot of lipids, carbohydrates, phenolic glycoside and amino acid and its derivative metabolites was decreased significantly, but the content was increased significantly for some metabolites including alkaloids, fatty acids, nuleoside and its derivative and phenolics metabolites (Fig. 9). Among the metabolites that increased significantly were substances associated with resistance to specific microorganisms (e.g. Hyperforin, Capric acid), acids (e.g. Hexacosanedioic acid), and some volatile gaseous substances (e.g. Myristic Acid ethyl ester) (Fig. 9). In addition, some metabolites with broad-spectrum antibacterial function were found among the significantly reduced metabolites. Based on KEGG enrichment analysis, these metabolites were significantly enriched in glutathione metabolism, ABC transporters, choline metabolism in cancer, protein digestion and absorption, and phenylpropanoid biosynthesis (Fig. S2b). These results indicated that melatonin regulated peanut root exudates or soil microbial components, thereby changing the soil environment, and these indicators were reflected in the results Fig. 4.

### 3.9. Correlation analysis between soil microbiome and soil metabolomics data

To further analyze the metabolite functions involved in soil microorganisms, we used MetOrigin to correlate soil microbial data with metabolomic data using humans as hosts. Results showed that soil bacteria unclassified_Subgroup_10 PMT processing compared to the relative abundance of PCK was reduced and were significantly positive correlated with most of the lipid also significantly lower (Fig. 10). However, PMT treatment compared to PCK unclassied *Gemmataceae*, uncultured soil bacterium, unclassied MND1, unclassied *Flavisolibacter* relative abundance increased significantly, which linked a significant increase in metabolite content (Fig. 10). These metabolites included six alkaloids (Flutamide, 4-butyl-2,5-dimethylthiazole, 2-Methylbenzothiazole, Enalkiren, Camptothecin, 1 - Methylpyrrolinium), five fatty substances (Arachidic acid, 2, 3 - Dihydroxycyclopentaneundecanoic acid, Myristic acid ester, α-hydroxy lauric acid,(9R,13R)-1a,1b-dihomo-jasmonic acid), 4 phenolic substances (Butyl salicylate, Isoeugenol benzyl ether,2,6-dihydroxybenzoic acid, Terephthalic acid), 1 amino acid (DL-Histidinol), 1 lipid (Beta-sitosterol), 1 other class (Chenodeoxycholic acid) (Fig. 10). However, the correlation analysis between fungi and metabolites indicated that only two fungi were associated with differential metabolites, while the abundance of these two fungi was not significantly different between PMT and PCK.

## 4. Discussion

Similar to plant hormone IAA, melatonin also can induce root growth and stimulate the formation of lateral and adventitious roots in plants (Arnao and Hernández-Ruiz, 2018). The root dry weight of peanut seeds

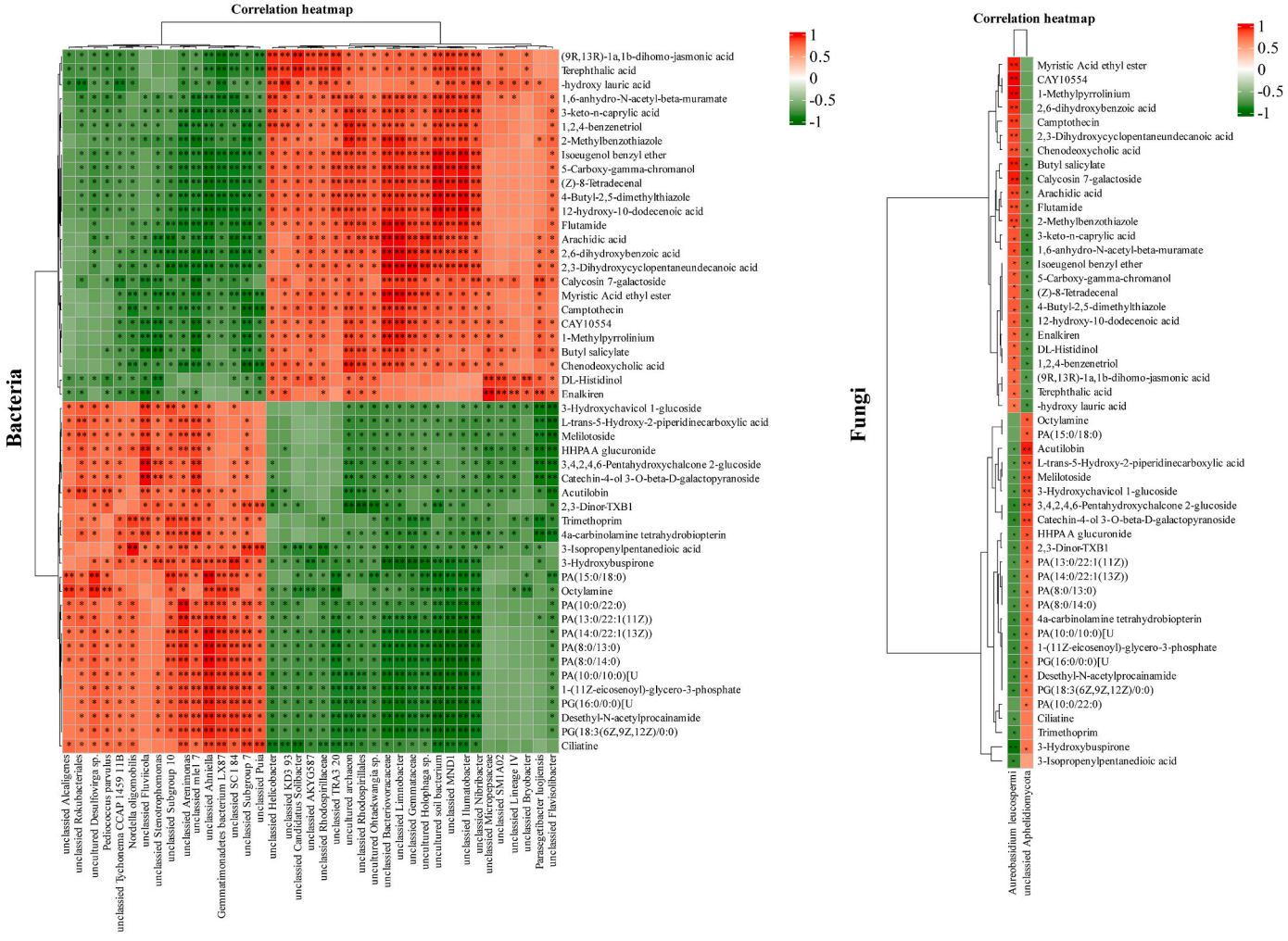


**Fig. 10.** Correlation analysis between soil microbiome and soil metabolomics data. Correlation analysis of soil microorganisms and soil metabolites. Red represents a significant positive correlation, and green represents a significant negative correlation. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

coated with melatonin significantly increased (Fig. 3e). With peanut plants growing and developing, formed roots also experience growth, senescence and death, and further new lateral roots produce and re-begin from growth to death. Phospholipids are important constituting parts of cell membranes, and their contents in the soil could reflect roots' senescence. We found significant and obviously lower phospholipids content in the soil under PMT treatment than the control (Fig. 9). These results indicated that melatonin not only promoted peanut root growth, but also delayed root senescence and prolonged its functional period.

Melatonin plays an important role in enhancing leaf photosynthesis and aboveground plant parts' growth and development (Sharma et al., 2020; Yang et al., 2022). Yang et al. (2022) proved that spraying exogenous melatonin on tea plants can improve the photosynthetic capacity. Melatonin added to the root systems can be absorbed by plants and then enriched in the leaves (Yoon et al., 2019). In our study, after melatonin treatment on peanut seeds, chlorophyll contents and nitrogen balance index were both significantly improved (Fig. 2b and c), indicating the nitrogen nutrition was obviously enhanced; meanwhile, electronic transport efficiency(Fig. 2d) and net photosynthetic (Fig. 2e) and were significantly increased. Those results are consistent with previous research. A recent research showed that melatonin seed priming improved early establishment of peanut cultivars (De Camargo Santos et al., 2024). Further, the number of peanut needles and immature pods were significantly increased by melatonin treatments (Fig. 3a and b), and that peanut yield and mature pod number were improved by same melatonin treatment (Fig. 4). Previous studies had shown that phytohormones play an important role in regulating peanut pod size, such as IAA, gibberellin (GA) and brassinosteroid (BR) (Wang et al., 2022). Furthermore, Melatonin enhanced maize seed weight by increasing growth-promoting content of phytohormones like zeatin + zeatin riboside, IAA and GA (Yoon et al., 2019). Considering IAA and GA can promote peanut peg development (Ahmad et al., 2021), we speculate that melatonin improves peanut peg development by upregulating GA and IAA contents in pegs.

In addition to the roles of phytohormone itself on plants, the friendly soil environment comprising of more available nutrient supply, more beneficial microbes and less toxic substances, plays an important role in plant growth and development as well as improving crop yield and quality in the ecological environment of plant growth. Melatonin not only made peanut root and aboveground parts develop better, but also created a friendly soil condition. A PMT treatment of the soil was found to increase the availability of ammonium nitrogen and phosphorus in the soil compared to a PCK treatment of the soil to promote the plant growth and development (Fig. 5a, b, c). In addition, the EC and pH of soil treated with melatonin (PMT) decreased compared with PCK (Fig. 5d and e), which may be related to melatonin promotes the absorption of ammonium nitrogen and other cations, meanwhile resulting in the release of a large number of hydrogen protons into the soil and changes the soil environment. Under uncultivated peanut conditions, melatonin treatment significantly reduced the nitrate nitrogen concentration in soil and significantly increased pH (Fig. 5b). Similarly, melatonin itself could increase the content of ammonium nitrogen and available phosphorus (Fig. 5a–c). That may be related to the change in the soil microbial community caused by melatonin itself. Soil microbial results proved that there were significant changes in microbial α diversity (Fig. S1) and relative abundance (Fig. 6a and b) under NPMT treatment compared with NPCK. Studies by Madigan et al. (2019) also revealed that melatonin could change the soil microbiota's community structure (Madigan et al., 2019).

The interaction between plants and soil microbes is well known to play an important role in creating a healthy living environment for plants and promoting their growth (Olanrewaju et al., 2019; Sun et al., 2021; Thakur et al., 2023). For example, some symbiotic nutritive fungi promoted plant root secretions, thus regulating plant growth and development environment (Reinhardt, 2007). Some saprophytic fungi degrade plant residues in soil, therefore increasing the content of organic matter in soil to promote plant growth and development (Medina et al., 2020). Based on our results, we found that the relative abundance of saprophytic functional fungi in PMT was significantly higher than that observed in PCK (Fig. 8a). However, there are also some pathogenic fungi in the soil. These fungi infect the plants, cause different diseases, and hinder the growth and development of the plants (Doehlemann et al., 2017). In our results, the relative abundance of pathogenic fungi decreased significantly under PMT compared with PCK (Fig. 8b). The result potentially proves that melatonin can regulate soil microbial community structure to inhibit the proliferation of pathogenic bacteria. A further analysis of soil microbial strains revealed a significant increase in the relative abundance of *Trichoderma* and *Penicillium* strains in PMT treatment when compared to PCK treatment (Fig. 7b). *Trichoderma* and *Penicillium* strains are capable of producing a variety of secondary metabolites, including antibiotics, to resist pathogen infection in plants (Perrone et al., 2017; Modrzewska et al., 2022). These results potentially demonstrated melatonin's role in improving peanut tolerance to biological stress.

Peanut growth and development are also influenced by a number of bacteria in crop growing soil that regulate the soil environment, improve crop tolerance to biological stress, and improve soil quality. For example, some chemotactic bacteria can colonize around crop roots using root exudates as signaling molecules (Feng et al., 2021). We found that the levels of gamma-glutamylisoleucine, L-histidine, phthalic acid, dehydroascorbic acid, guanine and arabinofuranosylguanine were significantly increased (Fig. 9). In previous studies, these metabolites were found to be direct chemoattractants of *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas putida* (Glekas et al., 2012; Fernández et al., 2016; Feng et al., 2018). The bacteria contribute greatly to the reduction of biological stress and the promotion of plant growth. As an example, *Bacillus amyloliquefaciens* is one of the best agents for biofertilizers and biocontrol agents in agriculture (Luo et al., 2022). The results of this study also showed significant increases in the relative abundance of some bacteria treated with PMT compared to control bacteria, such as the unclassified *Geminicoccaceae*, the *Nitrospirayu*, and the *Sphingomonas* (Fig. 6a, b, c, d). *Sphingomonas* bacteria are involved in soil restoration and production of growth-promoting plant hormones (Asaf et al., 2020), and *Nitrospirayu* bacteria contribute to the regulation of nitrate reduction (Daims et al., 2015; Daims and Wagner, 2018). In addition, we also found that PMT treatment compared with NPMT, the relative abundance of *Sphingomonas* was significantly increased (Fig. 6a and b). These results potentially proved that melatonin and peanuts jointly regulate soil microbial communities, maintain a good soil environment, and promote plant growth and development. Zhang et al. (2024) also confirmed that melatonin can improve microbial distribution and nutrient absorption, thus promoting the growth of kiwifruit. These are also reflected in an increase in ammonium nitrogen content of soil (Fig. 5a), which promotes the accumulation of plant biomass (Fig. 3).

In addition, the regulation function of melatonin in the soil microenvironment was also manifested in the interaction function of bacteria and fungi. We found that the content of some metabolites resistant to pathogenic fungi increased significantly in soil, such as chenodeoxycholic acid and capric acid (Fig. 9). In potato dextrose agar medium, chenodeoxycholic acid inhibited the growth of *Botrytis cinerea* and suppressed the growth of *Plasmopara viticola* (Boubakri et al., 2015). Capric acid showed resistance to *Candida* fungus (Khalandi et al., 2020). In plants, jasmonic acid and salicylic acid contain the main hormonal mechanisms that regulate the resistance of the plants to external pathogens and insects (Zhang et al., 2017a; Ding and Ding, 2020). In this study, we found that the contents of two hormone derivatives (9R, 13R)-1a,1b-dihomo-jasmonic acid and Butyl salicylate were significantly increased after melatonin treatment (Fig. 9). The correlation analysis between soil microorganisms and metabolites showed that bacteria and their metabolites, such as (9R, 13R)-1a, 1b-dihomo--jasmonic acid and butyl salicylate, were significantly correlated

(Fig. 10). In addition, some symbiotic arbustic fungi affected the enrichment of terpenoids in plants (Welling et al., 2016). The results of this study showed that the contents of some terpenoids such as hyperforin, were significantly increased under the regulation of melatonin (Fig. 9). Studies by Pia et al. also showed that hypericin has significant antibacterial activity (Pia Schiavone et al., 2014). Exogenous melatonin has also been shown to improve cucumber angular leaf spot resistance caused by *Pseudomonas syringae* pv. Lachrymans (Li et al., 2022). Some fungi-specific metabolite nivalenol decreased to a shallow level under melatonin treatment, indicating pathogenic microorganisms secreting it existed with few number or weakly active status (Fig. 9). Meanwhile, secondary metabolites, which usually produced more under abiotic or biotic stress, were reduced. In detail, plant defensins like cyclobrassinin sulfoxide, 6-methylsulfinylhexyl glucosinolate, and (S)-Spirobrassinin lowered substantially, a major portion of alkaloids and phenolics downregulated to a few percent extent under PMT treatment (Fig. 9).

## 5. Conclusion

The present study revealed that melatonin could regulate peanut root exudates to reshape soil microbiota structure and jointly create a suitable soil microenvironment, thereby promoting peanut growth and development and further increasing peanut yield.

## Declaration of competing interest

The authors have declared no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.109307.

## Data availability

Data will be made available on request.

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## Glossary

*AM:* Arbuscular mycorrhizal
*IAA:* Indole acetic acid
*JA:* Jasmonic acid
*SA:* Salicylic acid
*Pn:* Net photosynthetic rate
*PSII:* Photosynthetic system II
*OTU:* Operational taxonomic units
*NMDS:* Non-Dimensional Scaling
*CE:* Collision energies
*SCAN:* Full scan mode
*OPLS-DA:* Orthogonal partial least squares-discriminant analysis
*KEGG:* Encyclopedia of Genes and Genomes
*PMT:* Planting with melatonin-dressing seed
*PCK:* Planting with no-melatonin-dressing seed
*NPMT:* No indeed planting with only melatonin-dressing seed coat
*NPCK:* No indeed planting with only no-melatonin-dressing seed coat
*PERMANOVA:* Permutational Multivariate Analysis of Variance
*GC-MS:* Gas Chromatography-Mass Spectrometer
*UPLC-MS:* Super-high performance liquid chromatography-mass spectrum