

含有益生菌成分的种子处理剂浸种对食用百合根际土壤微生物区系的调控效应

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摘要: 【目的】播种前应用有益微生物浸种是兰州百合 (*Lilium davidii* var. *unicolor*) 栽培中尚未应用的一项新技术。我们利用新一代测序技术 (NGS) 研究这种浸种方法对真菌和细菌群落结构的影响。【方法】将百合鳞茎浸泡在一种含有有益微生物的种子处理剂 (SP 处理) 中 4 小时, 然后于 7 月种植在土壤中, 并于 9 月取样调查植物生长情况、根际层土壤理化性质和微生物群落结构。此外, 我们还利用 PICRUSt 和 FUNGuild 软件预测了细菌通路和真菌功能。【结果】在 SP 处理下, 真菌和细菌群落结构发生了显著变化, 同时土壤养分条件也得到了改善。值得注意的是, 优势微生物群落的相对丰度发生了显著变化, 如真菌 *Basidiomycota*、*Pseudeurotium*、*Cladophialophora*、*Microascus* 和 *Dactylonectria*; 以及细菌 *Proteobacteria*、*Chloroflexi*、*Ochrobactrium*、*Lysobacter* 和 *RB41*。微生物功能预测结果表明, 在 SP 处理下, 病原真菌 (包括植物病原体) 减少, 内生真菌和腐生真菌增加。在前 20 种代谢途径中, 与 CK 相比, 80% 在 SP 处理中上调。【结论】用有益微生物浸种能促进兰州百合球茎的生长。有益微生物在调节土壤微生物结构、增强内生真菌积累、减少病原体和改善土壤功能方面发挥了重要作用。此外, 研究还发现特定的微生物群参与了土壤健康的维护。

关键词: 食用百合; 第二代测序技术 (NGS); 种子处理; 真菌群落结构; 细菌群落结构; 预防

Seed treating agent containing Beneficial Microbial Regulated rhizosphere soil Microorganism Structure of Lanzhou lily

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Abstract: 【Background】The application of beneficial microbial seed soaking prior to sowing represents a novel technology that has yet to be employed in Lanzhou lily cultivation. We conducted an experiment to investigate the impact of this soaking method on the fungal and bacterial community structures using next-generation sequencing technology (NGS). 【Methods】Lily bulbs were soaked in a kind of seed treating agent containing beneficial microbial (SP treatment) for 4 hours, then planted in soil in July and sampled in September for investigation of the plant growth, and the rhizosphere soil physicochemical properties and the microorganism community structures. Furthermore, we utilized the software PICRUSt and FUNGuild to predict bacterial pathways and fungal functions. 【Results】Under SP treatment, there were significant alterations in fungi and bacteria community structures, accompanied by improved soil nutrient conditions. Notably, the relative abundance of dominant microorganism groups, such as the fungi *Basidiomycota*, *Pseudeurotium*, *Cladophialophora*, *Microascus*, and *Dactylonectria*; as well as the bacterial *Proteobacteria*, *Chloroflexi* and *Ochrobactrium*,

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Lysobacter and *RB41*, underwent notable changes. Microorganism function prediction results indicated a reduction in pathotrophic fungi (including the plant pathogens) and an increase in endophytic and saprotrophic fungi under SP treatment. Among the top 20 metabolism pathways, 80% were upregulated in SP treatments compared to the CK. 【 **Conclusions** 】 Seed soaking with beneficial microbial strain promoted growth of Lanzhou lily bulbs. The beneficial microorganisms played a crucial role in regulating soil microbial structures, enhancing the accumulation of endophytic fungi, reducing pathogens, and improving soil function. Furthermore, specific microbial groups were found to be involved in maintaining soil health.

Key words: edible lily; second-generation sequencing technology (NGS); seed treatment; fungal community structure; bacterial community structure; prophylaxis

Seed soaking with microbial agent before sowing is a kind of new seed treatment technology, which can promote seed germination as well as seedling growth status. During this process, microorganism can not only regulate seed water absorption, but also cover on the seed surface to protect seeds from pathogens infection^[1]. Many literature reported seed soaking with beneficial microorganism can improve seed germination rate, increase seed vitality, promote germination speed and guarantee germination quality^[2], such as maize seed soaking significantly improved the germination rate of maize in fields^[3]. Some reports focused on seed soaking effect on plant growth, seed soaking with beneficial bacteria (*Bacillus strains*) had antagonistic and competitive effects on plant growth promotion^[4-5].

In recent years, people pay much attention to the important role of plant rhizosphere microbial community composition in keeping soil health. Microorganisms in soil has much functions in the soil ecosystem by maintaining soil nutrient circulation, doing biological control of soil-borne diseases, and inducing plant disease resistance, and the microorganism abundance and diversity are closely related to plant species, soil types and cultivation methods^[6]. Many functional microorganisms have been extensively reported as a plant growth promoter on plant growth^[7], for examples the bacterial genus *Sphingomonas* plays an important role in the biological control of pathogenic bacteria^[8]; The phylum Basidiomycetes are associated with soil properties and vanilla wilt^[9]; the genus *Mortierella* has the function of dissolving phosphorus in soil, and can promote the increase of soil organic matter and nutrient content^[10]. However, at present, there are few reports on

the changes of plant rhizosphere soil microbial population after seed soaking. Therefore, it is of great significance to study the response mechanism of rhizosphere soil microorganisms to seed soaking treatment with microbial agents.

Lanzhou lily (*Lilium davidii* var. *unicolor*) is the only sweet lily in China. It is a kind of food lily and its bulb is consumed as vegetable. Compared to seed disinfection, seed treating agent with beneficial microorganism before sowing is safer to consumers. However, the lily seed soaking has not been used before. It is well known that microbial agent is very sensitive to soil environment, such as soil moisture, pH, temperature, etc. Our team used a kind of commercial microbial agents (named Special 8™, it contains 22 beneficial bacteria species) to alleviate Lanzhou lily replanting problems, and we found out it effective in this region, which promoted the seed germination, seedling growth, bulb yield and quality during its three years of cropping time from 2018 to 2020^[11-12]. In 2021, we repeat this experiment and observed similar results. Lanzhou lily propagates asexually with small bulbs (20–30 g), and the bulb is with root (about 1 cm) when it is sowed (Supplementary Fig. 1), which is helpful for the beneficial microorganism covering on the bulb or root surface. Moreover, we observed the bulb germinated early, which mean that the seedling development and the root exudates might be changed. Thus, we hypothesized that the rhizosphere microorganism must response to these dynamics at the seedling stage. But what the response was? How long would the change keep on? We knew nothing about it.

Based on this background, we carried on this

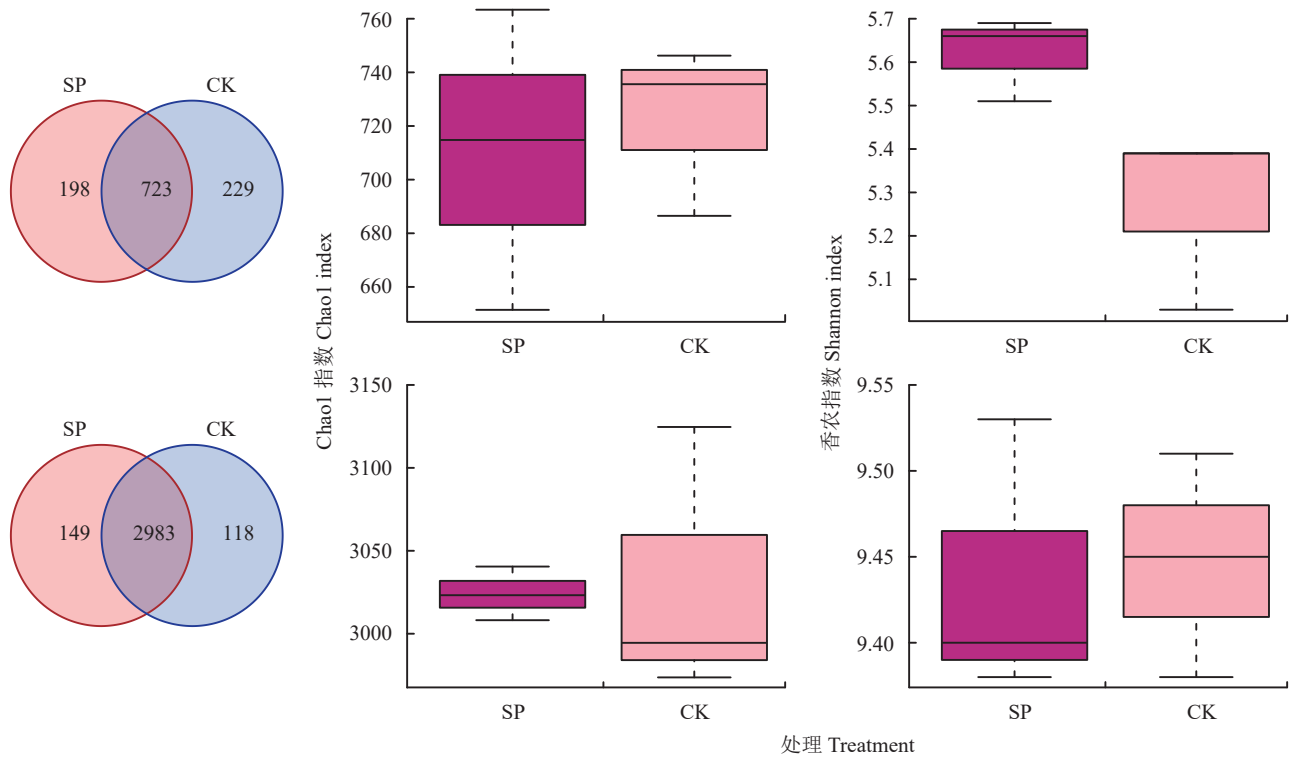
图 1 根际土壤 OUT 分布与 α 多样性分析

Fig. 1 The distribution and diversity analysis of rhizosphere soil OTU

(A): 真菌韦恩图 Chao1 多样性; (B) 真菌香农指数; (C) 细菌韦恩图 Chao1 多样性; (D) 细菌香农指数; 箱式图中的中间线表示数据的中位数, 箱体的上下框分别是数据的上四分位数和下四分位数, 箱体外上下短线分别代表数据的最大值与最小值。

(A): Fungus Venn diagram, fungus Chao1 index; (B) Shannon index diagram; (C) bacteria Venn diagram, bacteria Chao1 index; (D) Shannon index diagram; The middle line in the box plot represents the median of the data, the upper and lower frames of the box are the upper and lower quartile of the data, and the short lines above and below the box represent the maximum and minimum, respectively.

experiment in 2019 to research how the seed treating agent containing beneficial microbial regulated the lily microorganism structures, and collected the rhizosphere soil samples and analyzed both the fungal and bacteria community structure by next generation sequencing technology (NGS), and predicted the bacterial pathway and fungal function via the software PICRUSt and FUNGuild. The aims of this experiment were: 1) to find how the microorganism structures changed at the lily seedling staged under seed soaking treatment, and what are the key bacterial and fungal members related to soil health; 2) to elucidate the biological regulation mechanism with seed treating agents related beneficial microbial.

1 Materials and methods

1.1 Field description and experimental design

The sample site was located in Jiangjiashan village, Lintao County, Gansu Province (35°49'12"N,

103°53'13"E, 2330 m elevation), where is mountainous and semi-humid with a short frost-free period each year, very suitable for lily cultivation. The experiment field had been used for Lanzhou lily, and was fallow for one year before the experiment. The soil physiological and chemical characters was as following: organic matter 9.21 g/kg, alkali-hydrolyzable N 26.33 mg/kg, available K 47.96 mg/kg, available P 17.41 mg/kg, conductivity 1.22 mS/cm, pH 8.13.

We designed a randomized block experiment, which included two treatments: CK (no seed treating); SP (seed treating in solution containing microbial agents 5 mL/L). The test microbial agents Special 8™ was composed of 22 types of beneficial bacteria, and the main groups are *Bacillus*, *Sporolactobacillus*, *Pseudomonas*, a total of 15,000 cfu/g, produced by Qingdao Yuanhui Biological Environmental Protection Technology Co., LTD., Qingdao, China. In addition, the Special 8™ was added with 0.33 g/L KH_2PO_4 and 1.67 g/L root generation

powder (the main components are indoleacetic acid and sodium naphthalene acetate, Shandong Haidai Oasis biological engineering Co., LTD.). Three replicates were set for each treatment, and the plot area was 20 m² (10 m × 2 m). The bulbs weighed 17±2 g, were soaked for 4 hours, then dried in air and sowed into soil. The lily was sown at a density of 0.30 m × 0.15 m per plantlet in 18 April 2019. The field was managed with traditional soil managing practices in this region and without irrigation.

1.2 Sample collection and determination of the soil and plant growth indicators

1.2.1 Plant and soil sample collection Five-point sampling method were used to collect soil samples on 12 June 2019 (lily seedling stage). 4 plants randomly selected for each point, and a total of 20 plants were selected within each plot. Plants were gently shaken by hand to collect the soil adhering to plants roots, which was taken as rhizosphere soil samples. and the rhizosphere soils from the 20 plants were mixed to generate one soil sample. Each soil sample was divided into two parts, one was brought to the laboratory on dry ice and stored at - 80°C for downstream DNA extraction, and the other part was air-dried for soil characteristic detecting. And the 20 plants were brought into laboratory to determine the seedling growth indicators.

1.2.2 Determination of plant growth index The lily was sown in 18 April 2019, the germination potential and germination rate were determined at 30 days and 36 days after planting, respectively. Germination potential (%) = Germinated bulbs at 30 days/total bulbs seed × 100%. Germination rate (%) = Germinated bulbs at 36 days after planting/total planted bulbs × 100%. The stem diameter and plant height were measured, and the seedling root activity was measured by TTC method described in our published work^[13].

1.2.3 Determination of soil physicochemical properties

Soil organic matter was determined by potassium dichromate method, alkali-hydrolyzable nitrogen was determined by alkali hydrolyzed diffusion method, available P by molybdenum antimony colorimetry, available K by NaNO₃-sodium tetraphenylborate turbidity method, conductivity by EC Meter (DDS-307A Shanghai Leidian) and pH by 1:5 soil: water [w: v] extraction-pH

Meter (PHS-3E; Shanghai Jingke), respectively^[14].

1.3 High-throughput sequencing by ILLUMINA Miseq

DNA extraction and PCR amplification were conducted as described in our published article^[12]. During PCR amplification, the V3-4 high variant region of the bacterial 16S rRNA gene was generated by forward primer 338-F (5'-ACTCCTACGGGAGGCAGCAG-3') and reverse primer 806-R (5'-GGACTACHVGGGT-WTCTAAT-3'), and the fungal amplicon was generated by forward primer ITS1-F (5'-CTTGGTCATTTAGA-GGAAGTAA-3') and the reverse primer ITS2-R (5'-TGCGTTCTTCATCGATGC-3') were generated at the Allwegene Company's (Beijing) Miseq platform for deep sequencing. After the run, image analysis, base calling, and error estimation were performed using Illumina Analysis Pipeline Version.

1.4 Data analysis

Taxonomy of 16S sequences was performed according to the SILVA ribosomal RNA gene database^[15] and Greengene[®] and classification of ITS1 sequences was performed according to the UNITE database pair. OTU Venn diagram, Alpha diversity Index and Principal Coordinates Analysis (PCoA) were used to analyze the diversity of microbial communities at the OTU level. Linear Discriminant Analysis Effect Size (LEfSe) method was used to detect species with significant abundance differences between treatments, and the threshold was set at 3.0. At the genus level (the first 15 genera), a redundancy analysis (RDA) method was used to reveal the effects of soil physicochemical characteristics factors on the formation of microbial communities. The fungal function prediction analysis was via the software FUNGuild, and the bacterial function prediction analysis was via the software PICRUST^[17].

Microsoft Excel 2010 software was used for raw data processing and graphing, SPSS 22.0 statistical software for ANOVA, and Duncan's multiple comparison method was used to detect significant differences ($P < 0.05$).

1.5 Accession Numbers

The sequences obtained in this study were submitted to the NCBI Sequence Read Archive (SRA) under the

Bioproject ID PRJNA736164.

2 Results

2.1 Lily seedling growth under the SP treatment

Seed bulb germination quality and the seedling growth indicators increased signally under SP treatment. When compared to CK, in SP treatment, the germination rate signally increased by 18.05%, and the root activity increased by 32.67% (Table 1).

2.2 Rhizosphere soil physicochemical properties under SP treatment

Soil nutrition and physiological indexes were signally changed under SP treatment. Compared to CK, available P and alkali-hydrolyzed N in SP treatment were signally increased by 127.83% and 55.25%, respectively, pH and conductivity were signally decreased by 2.47% and 6.41%, respectively (Table 2).

2.3 Overall structural changes of microorganism communities in rhizosphere soil

Alpha diversity indices were evaluated based on OTUs. For fungi, the OTU distribution revealed a total of 1150 OTUs and 198 and 229 unique OTUs in SP and CK, respectively (Fig. 1A). As for bacteria, the OTU distribution revealed a total of 3250 OTUs and 149 and 118 unique OTUs in SP and CK, respectively (Fig. 1C). Statistical analysis revealed both the Chao1 index and Shannon index (in fungi and bacteria) SP treatment did

not significant change when compared to CK ($P>0.05$) (Fig. 1BD).

Beta diversity was evaluated by PCoA based on all the microorganism OTUs. The result successfully represents the sample data, the microorganism in SP treatment clearly separated from CK. For fungi, the first principle component axis (PCoA1), which contributed 34.30% of the total variation, and the second principle component axis (PCoA2), which contributed 27.33% of the variation, explained 61.63% of the variation (Fig. 2A). As for bacteria, the first principle component axis (PCoA1) and the second principle component axis (PCoA2) totally explained 52.82% of the variation (Fig. 2B).

2.4 Overall structures of fungi and bacteria under SP Treatment

A large number of bacterial and fungal groups were identified at all taxonomic levels. RDA revealed that soil bacterial and fungal community structure was strongly affected by the changes of soil properties under seed soaking treatment (Fig. 3AC). At the phylum level, the two axes of the RDA explained 58.55% and 20.19% of the total variation in soil fungi data (Fig. 3A), and 67.05% and 15.56% of the total variation in soil bacteria data (Fig. 3C), respectively.

For fungal, the dominant phyla were Ascomycota, Mortierellomycota and Basidiomycota, with average RA values of 71.73%, 14.82% and 2.84%, respectively.

表 1 SP 处理下兰州百合种苗的生长情况

Table 1 Lanzhou lily seedling growth status under the SP treatment

处理 Treatment	发芽率 (%) Germination rate	发芽势 (%) Germination potential	株高 (cm) Plant height	茎粗 (mm) Stem diameter	根系活力 (mg/g·h) Root activity
CK	0.72±0.03 b	0.46±0.11 ab	15.93±0.31 b	5.85±0.31 b	289.11±2.02 b
SP	0.85±0.04 a	0.60±0.16 a	18.14±0.40 a	6.48±2.1 a	383.56±3.01 a

注: 不同小写字母表示处理间差异达到5%水平。

Note: Lower case letters indicate significant differences at the 0.05 level for each trait under different treatment conditions.

表 2 根际土壤理化性质

Table 2 Rhizosphere soil physicochemical properties under SP treatment

处理 Treatment	pH	有效磷 (mg/kg) Available P	电导率 (mS/cm) Conductivity	有机质 (g/kg) Organic matter	碱解氮 (mg/kg) Alkali-hydrolyzed N	有效钾 (mg/kg) Available K
CK	8.11±0.00 a	21.45±0.04 b	0.78±0.02 b	9.43±0.54 ab	29.88±1.64 b	200.46±4.63 a
SP	7.91±0.03 b	48.87±2.63 a	0.73±0.01 a	9.89±0.42 a	46.39±1.30 a	266.61±1.78 a

注: 不同小写字母表示处理间差异达到5%水平。

Note: Lower case letters indicate significant differences at the 0.05 level for each trait under different treatment conditions.

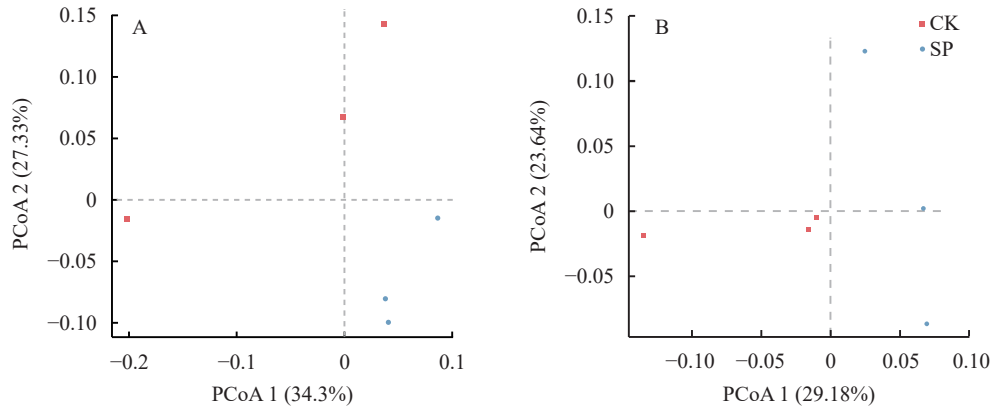


图 2 根际土壤真菌 (A) 和细菌 (B) PCoA 分析

Fig. 2 The rhizosphere soil fungi PCoA (A) and bacteria PCoA (B) after Lanzhou lily seed soaking treatment

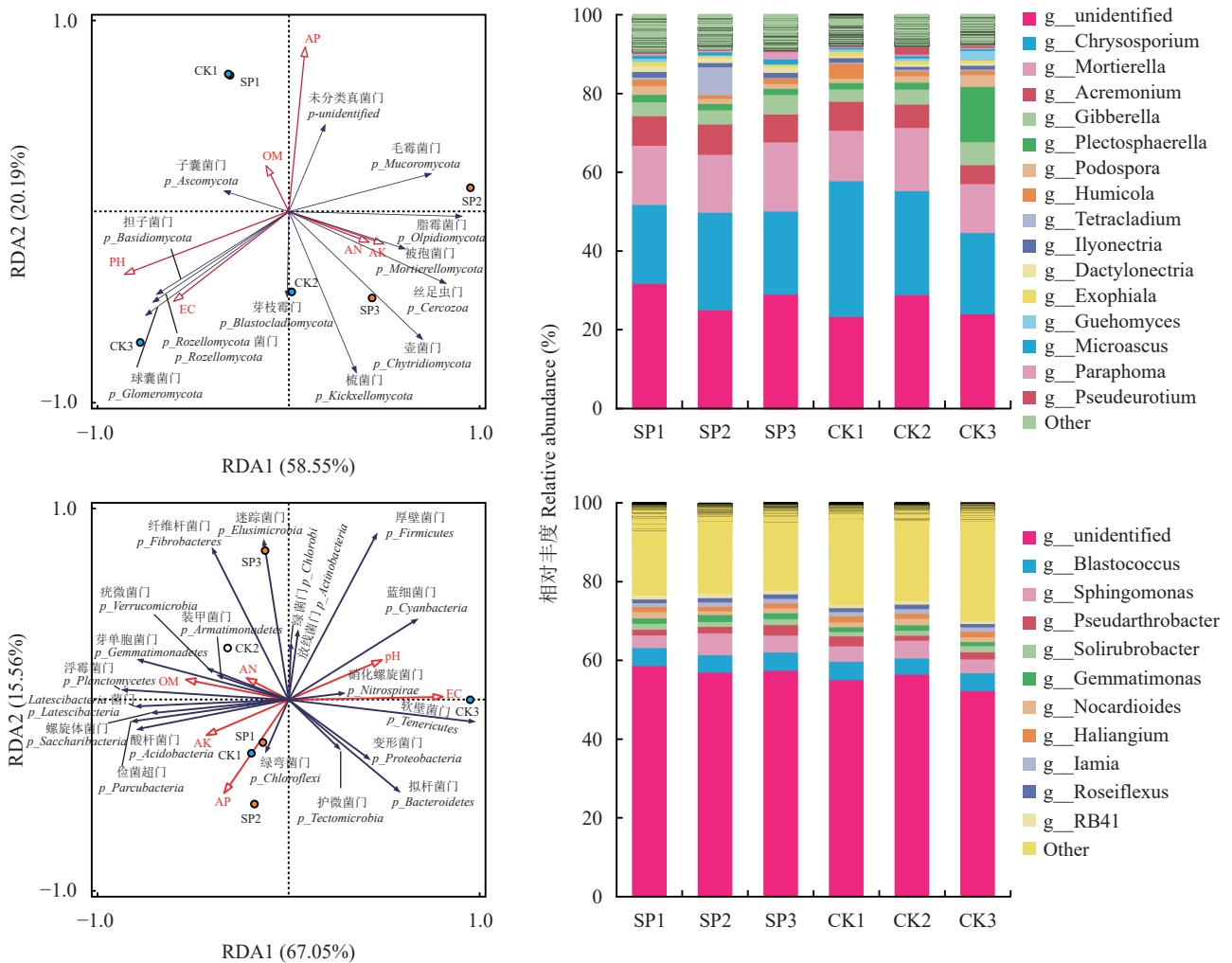


图 3 根际土壤微生物结构全分析

Fig. 3 Overall microbial community structures after seed bulb soaking of Lanzhou lily

注: (A)、(C) 前 20 位真菌门、细菌门冗余分析 (B)、(D): 丰度前 15 位真菌属、10 位细菌属组成图。

Note: (A)、(C) Redundancy analysis (RDA) of top 20 fungal phyla and top 20 bacterial phyla (B)、(D): Bar plots of top 15 fungal genera and top 10 bacterial genera.

The sum of these three fungi accounted for 88.55% and 90.23% of the total fungi number of SP and CK

respectively. At phyla level, 10.92% and 9.31% of SP and CK could not be identified, respectively. The

dominant genera were *Chrysosporium*, *Mortierella*, *Acremonium*, *Gibberella*, *Plectosphaerella*, *Podospira*, *Humicola*, *Tetracladium*, *Ilyonectria* (average RA>1%). The sum of the above dominant genera accounted for 57.61% and 62.67% of the total fungi number of SP and CK, respectively. *Dactylonectria* showed significant difference between the two treatments, SP increased 95.21% compared to CK. At genus level, 28.75% and 25.53% of SP and CK could not be identified, respectively (Fig. 3B).

For bacteria, the dominant phyla were Actinobacteria, Proteobacteria, Gemmatimonadetes, Chloroflexi, Acidobacteria and Bacteroidetes, with average RA values of 34.84%, 34.75%, 10.26%, 8%, 7.97%, 6.03% and 2.86%, respectively. The sum of the six bacterial accounted for 96.56% and 96.88% of the total bacterial number of SP and CK respectively. Among them,

Proteobacteria showed significant difference between the two treatments, and SP decreased 10.06% compared to CK. The dominant genera were *Blastococcus*, *Sphingomonas*, *Pseudarthrobacter*, *Solirubrobacter*, *Gemmatimonas*, *Nocardioides*, *Haliangium* and *Iamia* (average RA>1%). The sum of the above dominant genera accounted for 17.32% and 16.71% of the total bacterial number of SP and CK, respectively (Fig. 3D).

2.5 The specific fungal and bacterial groups under SP treatment

LEfSe analysis showed the signally changed groups at different taxonomic levels under SP treatment (Fig. 4A and 4B). The results showed that some important fungal groups signally dominated in SP treatments. There were 16 distinct groups of fungi in different treatments (Fig. 4A). The phylum Basidiomycota and its member genus *Pseudeurotium* decreased signally in SP treatment

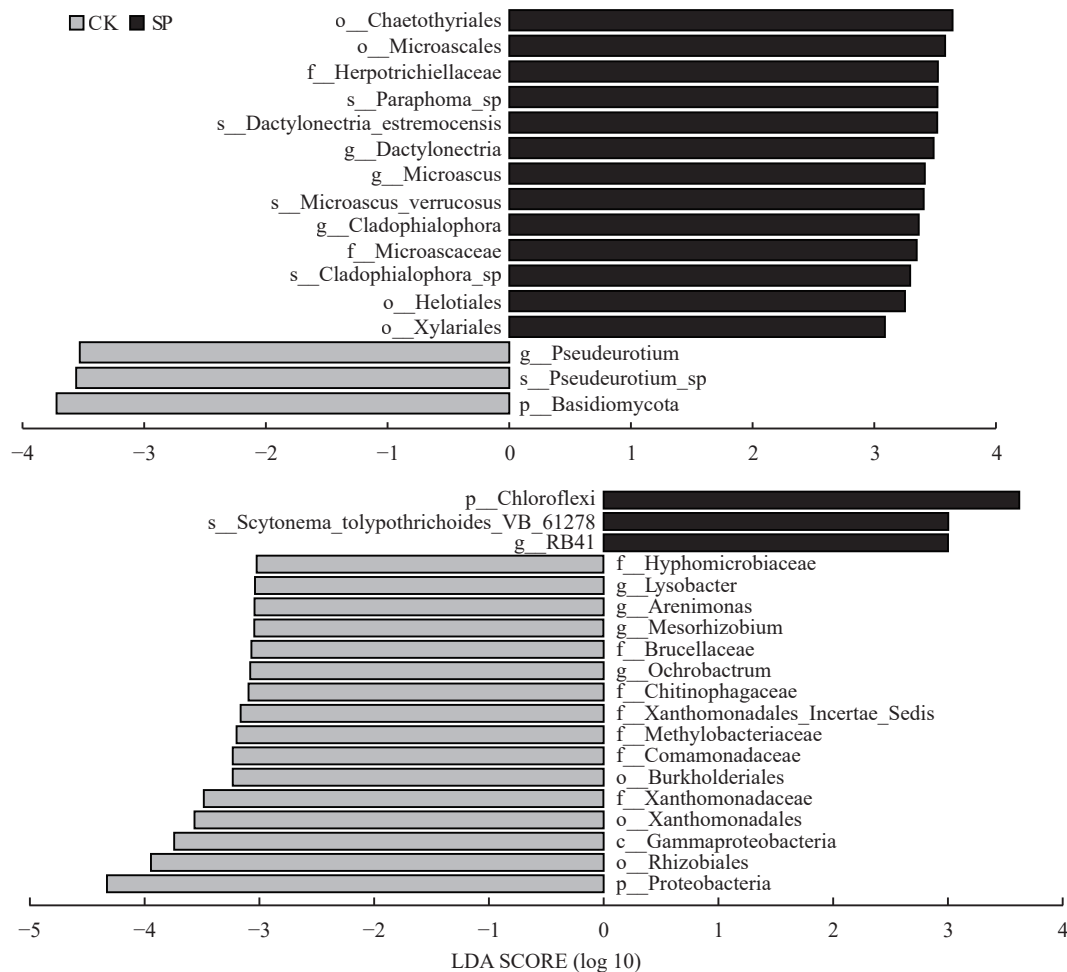


图 4 根际真菌 (A) 细菌 (B) 相对丰度 LEfSe 分析结果

Fig. 4 The LDA obtained from the LEfSe analysis shows the relative abundance of fungi (A) and bacterial (B) communities in the rhizosphere soil after seed soaking in Illumina Miseq Lanzhou lily

when compared to CK, while the genera *Dactylonectria*, *Microascus* and *Cladophialophora* increased signally. There were 19 distinct groups of bacteria in different treatments (Fig. 4B). We listed some importantly and signally changed bacterial genera: Compared to CK, the phylum Proteobacteria and its member genera *Ochrobactrum*, *Lysobacter*, *Arenimonas* and *Mesorhizobium* decreased signally in SP treatment, while the phylum Chloroflexi and its one member *Scytonema_tolypothrichoides_VB_61278*, *RB41* signally increased. In addition, Among the dominant genus (average RA $\geq$ 0.5%), *Penicillium*, which is widely reported beneficial to soil health, signally increased in SP treatment when compared to CK (Supplementary Fig. 2).

2.6 Fungi and bacteria function prediction

For fungi, according to basic trophic types, FUNGuild function prediction analysis divided them into three types: Symbiotroph, Saprotroph, Pathotroph. Compared with CK, the Pathotroph fungi, which destroyed the host cells, decreased by 2.86% in the SP treatment, and the Saprotroph fungi and Symbiotroph fungi increased by 5.43% and 35.82% (Fig. 5A). Further classification showed that there were 15 guilds with different functions. Among them, we listed three guilds with important functions related to soil health of plant growth. Compared with CK, the Endophyte increased by 14.47% in SP treatment, while the Plant Pathogen decreased by 0.64% (Fig. 5B).

For bacteria, PICRUST2 function prediction showed that among the top 20 metabolism pathway, 80% metabolism pathway in SP treatments regulated up when compared to CK, and some metabolism pathway, such as activity of Cell cycle-Caulobacter, Alanine and aspartate and glutamate metabolism, D-Alanine metabolism were signally enhanced (Fig. 5C).

3 Discussion

In this experiment, we used a kind of seed treating agent that contains commercial microbial agents (Special 8™, which contains 22 types of beneficial bacteria, a total of 15,000 cfu/g) to treat the lily seed bulb. As we mentioned above, our team proved that this commercial microbial agents was effective in the lily production area

(where it is cold in spring, the soil is dry and alkaline)^[11-12], and the bulb had root about 1 cm long when it is sowed (Supplementary Fig. 1), which is helpful for the beneficial microorganism covering on the bulb or root surface, and facilitate cloning. Thus, we believed that during this bulb soaking process, the beneficial microorganisms must covered on the seed surface, propagate quickly, and protect the bulbs from pathogens infection. For Lanzhou lily, the bulb seed needs long time to germinate (about one and a half months) and to generate new roots (about two months late), so we added KH_2PO_4 and commercial root generation powder as auxiliary ingredients for the seed treating agent, which might play significant roles in changing soil physicochemical indexes and promoting plant growth. However, the beneficial microorganisms (from Special 8™) played the most important role in altering the microbial structure during at the lily seedling growth period.

In this study, both the fungal and bacterial Alpha diversity did not significantly change, but the Beta diversity significantly changed in the SP treatment: PCoA analysis showed that CK and SP treatments were significantly separated, and they explained 61.63% of the variation for fungi (Fig. 2A) and 52.82% of the variation for bacteria (Fig. 2B). This study also showed that soil physiological indexes changed and the soil nutrition level improved in the SP treatment (Table 2). Additionally, the soil physiological characters made significantly contributions to the construction of microorganism structures (Fig. 3AC). Numerous researches have reported the close relationship between soil biological and physicochemical characteristics, including our previous research, which proved that soil physicochemical characteristics impact the construction of fungal structures^[17] and bacterial structures^[18] in the lily rhizosphere soil.

LEfSe analysis revealed some specific microorganism groups played important roles in maintaining soil health or contributing to plant growth. For fungi. The phylum *Ascomycota*, *Mortierellomycota* and *Basidiomycota* were dominant in the rhizosphere soil of lily. We believed the genus *Penicillium* is an important microbial group with positive effects on soil health. This genus signally increased under SP treatment (RA = 0.57%) when

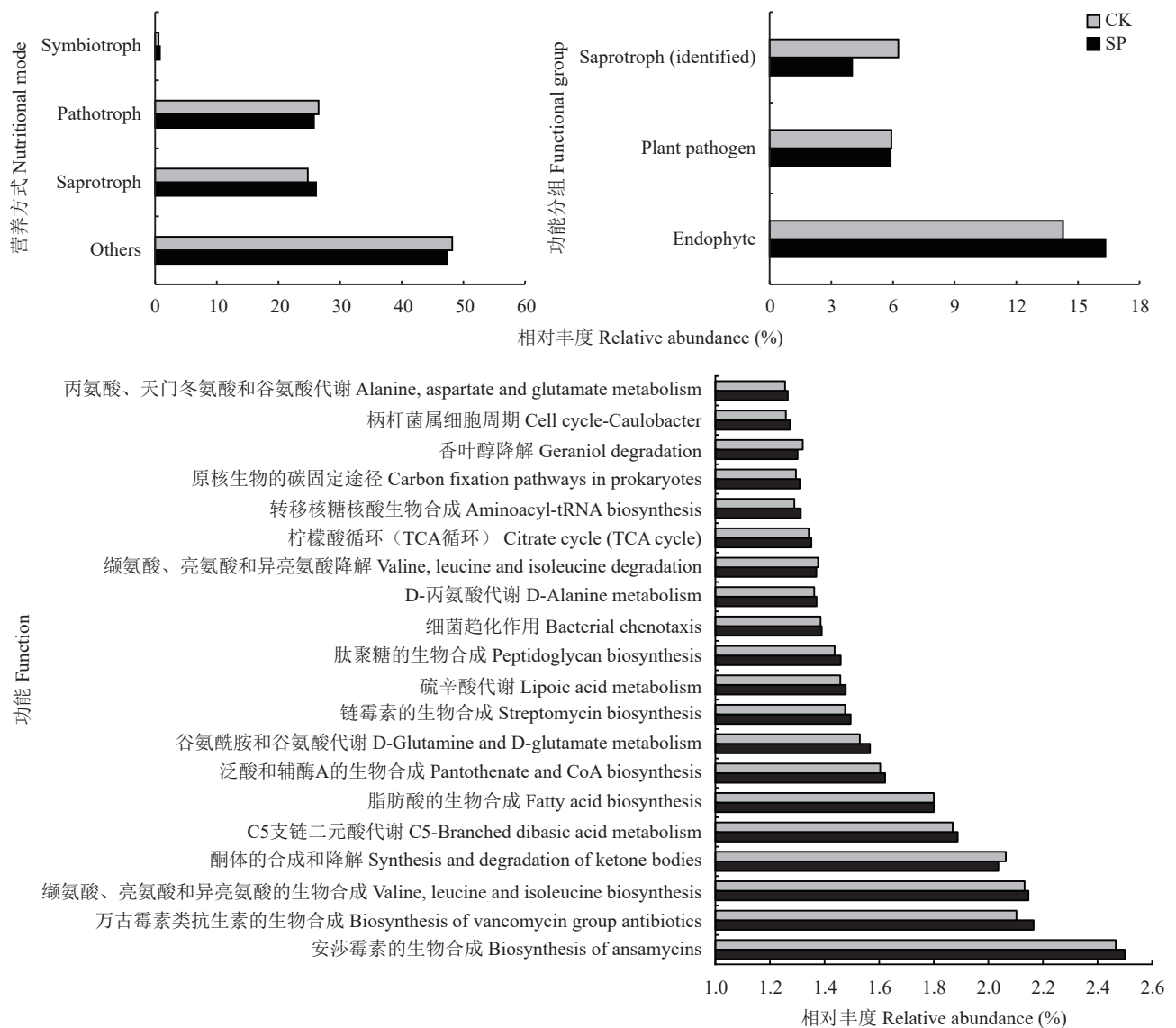


图 5 不同处理真菌细菌功能预测

Fig. 5 Fungal and bacterial functions prediction under different treatments

真菌功能用: Fungi function by FUNGuild prediction for three nutrient types (A), three important guilds from further classification data by FUNGuild prediction (B); Bacterial function prediction by PICRUSt2 for top metabolism pathway (C): Cell cycle-Caulobacter, Alanine and aspartate and glutamate metabolism, D-Alanine metabolism and Citrate cycle were significant differences in between treatments ($P < 0.05$).

compared to CK (RA = 0.36%, Supplementary Fig. 2). *Penicillium* can produce a variety of bioactive compounds, which can be used as fungal antagonists and plant growth promoters^[19]; and another reports showed the seed coated with *Penicillium* had comprehensive effect on seed disinfection, as well as improving seedling emergence rate and seedling vitality⁰. The phylum *Basidiomycota* might also be related to soil health. It was reported that *Basidiomycota* was related to soil properties and the occurrence of vanilla wilt disease^[8], and in this study, this phylum and its member genus *Pseudeurotium* decreased significantly under SP treatment (Fig. 4A). We

also found that the genus *Microascus*, *Dactylonectria*, and *Cladophialophora*, belonging to the phylum Ascomycota, increased significantly after SP treatment (Fig. 4A), but we have not referred to any literatures related to their functions on soil health.

For bacteria, the phyla *Actinobacteria*, *Proteobacteria*, *Gemmatimonadetes*, *Chloroflexi* and *Acidobacteria*, which are common in nature^[19], were dominant in rhizosphere soil of lily. This result supported the previous research conclusion on the dominant bacterial communities in rhizosphere soil of Lanzhou lily^[18]. We believed that the phylum *Chloroflexi* was a key microbial

group that was closely related to soil nutrition or the plant's nutrition utilization. LEfSe analysis showed that *Chloroflexi* and its member *Scytonema_tolypothrichoides_VB_61278* significantly increased in SP treatment (Fig. 4B). *Chloroflexi* prefers to live in an environment with sufficient nutrition^[20], and in this study, the soil nutrition level was improved under SP treatment (Table 2). We also found the phylum *Proteobacteria* and its member genera *Ochrobactrum*, *Lysobacter*, *Arenimonas* and *Mesorhizobium* significantly decreased under SP treatment. In our previous research, we proved the phylum *Proteobacteria* and its genus *Sphingomonas* were indicators of soil quality, and their abundance was positively correlated with the lily soil health^[18]. However, *Sphingomonas* did not significantly change under SP treatment in this research. Thus, the results in this experiment seem not consistent with our previously conclusion. *Proteobacteria* is very sensitive to environment. We speculated that the departure in results may be due to soil sample collection. The two field experiments were conducted in the same village, but the soil sample collection times were different. The previous research's soil sample was collected in July at the lily plant's flowering stage, whereas this research's soil sample was collected in June at the lily's seedling stage.

The results showed that SP treatments not only increased bulb seed germination, but also altered microorganism structures in the seedling stage. As we mentioned above, Lanzhou lily is a type of asexual propagating plant, small bulbs are used as seeds. Before sowing, the roots are usually trimmed to a length of 1 cm to facilitate the sowing practice (Supplementary Fig. 1). Therefore, during the seed soaking process, the microbial agent can adhere to the wounded roots, which is helpful for microorganism to cover and infect the root or bulb surface and propagate in soil. On the other hand, under the SP treatment, the seed germinated earlier, leading to changes in root morphology and physiological state when compared to CK. These changes resulted in differences in root exudate secretions and formed a new type of selection pressure for the microorganisms. All these shift subsequently impacted the microorganism community structures and soil physicochemical properties, as well as their interaction^[21]. This description provided a reasonable

explanation for why bulb seeds soaked with microbial agents (before sowing) could induce significant changes in the soil microorganism in the seedling stage.

We are excited to find out that the endophyte fungi significantly increased under SP treatments (Fig. 5B). Among various fungal lifestyles, endophytic behavior stands out due to a series of benefits it provides to the host plants^{[22][26]}. Endophytic fungi live inside plant tissues without causing apparent diseases^[27,30]. These microorganisms establish a mutualistic ecological system in which both parties benefit from the interaction^[26]. In this experiment, a great number of endophytic fungi in the SP treatment were hosted in the lily tissues, requiring protection and nutrients from the lily plants. In return, these fungi contributed to the lily plant's growth and nutrient uptake. Furthermore, fungi function prediction also revealed that after SP treatment, there was a reduction in pathogenic fungi (especially pathogens) damaging the host cells, and an increase in saprotrophic fungi benefitting soil organic nutrition enrichment, which greatly contributed to soil health promotion (Fig. 5AB). Bacteria function prediction by PICRUST2 also supported this conclusion: 80% of the metabolism pathways in SP treatments were upregulated when compared to CK (Fig. 5C), and the root activity was also enhanced under SP treatment (Table 1).

In summary, this experiment confirmed our hypothesis: SP treatments could induce response in microorganism in the rhizosphere soil at the lily seedling stage and some specific microorganism groups contribute to maintaining soil health or promoting plant growth. Moreover, its influence lasted for a long time and ultimately improved the yields and quality of food lilies (results will be published in another article). In addition, it is reasonable to consider other microorganism agents (not only Special 8™, which was used in this study) in practical application. However, here we would like to emphasize the importance of choosing suitable microorganism agents, as different commercial products have different qualities and ingredients. Furthermore, it is crucial to maintain sufficient soaking times (for example, 4 hours of soaking in this experiment) to ensure that beneficial microorganisms can coat the bulb or root surface.

4 Conclusions

Bulb seed soaking with the seed treating agent (containing beneficial microbial) is effective for Lanzhou lily seedling growth. It can promote bulb seed germination and seedling growth. Among its main ingredients, the beneficial microorganisms play important roles in regulating soil microorganism structures, both the accumulation of endophytic fungi and the depletion of pathogens play positive roles in enhancing soil function, and some specific microorganism groups, such as the fungal phylum *Basidiomycota*, the genus *Penicillium* and the bacterial phylum *Chloroflexi*, are involved in maintaining soil health.

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Appendix

Supplementary Fig. 1. Picture shows the Lily seeds bulb (17±2 g) with long roots: the Lily seeds bulb with



roots was cut off (1 cm left) before sowing
Supplementary Fig. 2. The Relative abundance of *Penicillium* in different treatments

