The Physiological and Biochemical Effects of Phthalic Acids and the Changes of Rhizosphere Fungi Diversity under Continuous Cropping of Lanzhou Lily (*Lilium davidii* var. *unicolor*)

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Abstract. The autotoxicity of root exudates and the change of rhizosphere soil microbes are two important factors that affect the quality and yield of Lanzhou lily (*Lilium davidii* var. *unicolor*). Phthalic acid (PA) is a major autotoxin of the root exudates in Lanzhou lily. In this study, we treated plants with different concentrations of PA from the Lanzhou lily root exudates and then analyzed the effects of autotoxins on fresh weight, shoot height, root length, and Oxygen Radical Absorbance Capacity in root. The diversity of soil fungi in Lanzhou lily soil was analyzed using MiSeq. The results showed that PA induced oxidative stress and oxidative damage of Lanzhou lily roots, improved the level of the membrane lipid peroxidation, reduced the content of antioxidant defense enzyme activity and the nonenzymatic antioxidant, and eventually inhibited the growth of the Lanzhou lily. We found that continuous cropping of Lanzhou lily resulted in an increase in fungal pathogens, such as *Fusarium oxysporum* in the soil, and reduced the size of plant-beneficial bacteria populations. The results in this study indicate that continuous cropping would damage the regular growth of Lanzhou lily.

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Continuous cropping can cause soil-borne disease and crop autotoxicity, resulting in a decline in crop quality. Succession cropping leads to the changes of physical and chemical properties in soil that crops need to grow (Dou et al., 2016). In addition, changes in microbial community composition and the residue of the toxic substances following continuous cropping are also important factors causing the continuous cropping obstacle (Kennedy and Smith, 1995; Zhang et al., 2008). One of the obvious signs of soils with continuous cropping is that the soil is transformed from “bacterial” to “fungal,” and fungi are the main pathogens of plant disease (Ibekwe et al., 2002), including *Fusarium*, *Rhizoctonia*, *Pythium*, *Cylindrocarpon*, and *Phytophthora* (Mazzola, 1998).

Lanzhou lily (*Lilium davidii* var. *unicolor*) is an important economic crop in northwest China's Ningxia and Gansu provinces; however, continuous cropping has seriously affected the yield and quality of Lanzhou lily bulbs. *Fusarium oxysporum* is the main pathogenic fungus of Lanzhou lily. *Fusarium moniliforme*, *Fusarium tricinctum*, and *Fusarium solani* can also result in wilt disease (Shang et al., 2014). In addition to the impact of microorganisms, the toxic effects of root exudates cannot be ignored. Many studies have shown that the autotoxicity of root exudates is an important obstacle to continuous cropping. Different concentrations of root exudates have a significant effect on the growth of seedlings of Lanzhou lily. The low-concentration root exudates promote seedlings, whereas high-concentration root exudates repress seedlings (Chen et al., 2016). Studies have shown that PA can accumulate in soil with the increase in duration of monoculture, and PA may be one of the major factors inducing continuous cropping obstacles in Lanzhou lily (Wu et al., 2015). The aim of this study was to investigate the effects of continuous cropping on soil-borne disease and the crop autotoxicity in Lanzhou lily cultivation.

Material and Methods

Experimental materials and sample plot profiles. A total of 100 Lanzhou lily seed bulbs used in physiological experiments were purchased from lily dealers (Xiguoyuan of Lanzhou City, Gansu Province, China). The seed bulbs were dormancy broken through refrigeration for 60 d at 4 °C. PA was purchased from Tianjin Kexin Chemical Company (Tianjin, China). The test soils were obtained from the surface soil (0 to ≈15 cm) where lilies were grown. After removing the stones of soils by sieving through a 4-mm mesh screen, the soil was mixed evenly and used as the growth medium for Lanzhou lily.

Soil samples for fungal diversity analysis were collected from sites in Xiguoyuan of Lanzhou City, Gansu Province, China (lat. 35°98'N, long. 103°78'E) that had grown lily for many years. Test soils came from different sites, including sites that planted lily for a year (RS1), 2 years (RS2), 3 years (RS3), and a site that was planted with lily and idle for a year (RS0). The experimental plot size was 100 × 100 cm, and there were 36 bulbs per plot. All plots were adjacent to each other to eliminate the variation of soil properties caused by spatial differences. There were three replicate plots in every site. We collected rhizosphere soil from four plants in every plot. Meanwhile, the fertilization program and the planting management measures were consistent in all plots.

Experimental designs. At the beginning of April, we used a pot experiment to investigate the effect of PA on growth of Lanzhou lily. Lily bulbs were immersed in Imazalil for 1 h to sterilize the surface, and then rinsed with tap water several times before planting;

3-cm lily bulbs were transplanted to the culture basin. Different concentrations of PA solution were used to water soil before planting at 0 (CK), 0.01, 0.05, 0.25, 0.5, and 1 $\mu\text{mol}\cdot\text{g}^{-1}$ (Wu et al., 2015). Each concentration was replicated in three basins, and each basin was planted with five bulbs. During cultivation, plants were watered every 2 days. To maintain soil moisture, culture basins were weighed to determine the amount of water, and the moisture was kept constant at $\approx 50\%$ of the maximum water-holding capacity. Hoagland nutrient solution was used for half a month to maintain the mineral elements needed for the growth of lily. All the culture basins were cultured in a plastic greenhouse for ≈ 75 d before reaching the blooming stage. At 75 d after sowing, the root length (cm), plant height (cm), and fresh weight (g) of the lily were measured. Lanzhou lily root and leaf were put into the liquid nitrogen and brought into the refrigerator [temperature (T) = -80°C] for physiological and biochemical analysis.

Because lily disease usually occurs in the late growth period of the plant, the sampling time of the continuous cropping rhizosphere soil of Lanzhou lily for the study of fungal diversity was determined in the lily bud stage to avoid the influence of disease on fungal community structure in rhizosphere soil of Lanzhou lily. In four different types of plots, which include plots that continuously crop Lanzhou lily 0 year (RS0), 1 year (RS1), 2 years (RS2), and 3 years (RS3), respectively. Three different communities were delineated for each plot. In each plot, the continuous cropping rhizosphere soils of four plants were collected and then the soil was mixed to form a single soil sample. Therefore, a total of 12 rhizosphere soil samples were obtained. The samples were placed in sterile plastic bags, put into the ice box for transport back to the laboratory, and then saved in the refrigerator (T = -80°C).

Analysis of physiological and biochemical effects of autotoxicity on Lanzhou lily. SOD (superoxide dismutase), POD (peroxidase), CAT (catalase) activity, H_2O_2 , MDA (Malondialdehyde), total phenol content, and hydroxyl radical scavenging ability were determined by kits (Suzhou coming biological technology co. LTD, Suzhou, Jiangsu Province, China). Methods were applied according to the instructions of kits. The root activity of the 5-cm root tip was determined using the 2, 3, 5-triphenyltetrazolium chloride method (Lindstrom and Nystrom, 1987). Chlorophyll (chlorophyll A and chlorophyll B) and carotenoids were extracted with 80% acetone and their contents (mg/g fresh weight) were determined according to the method of Lichtenthaler and Wellburn (Hartmut, 1983).

Fungal diversity in continuous cropping rhizosphere soil of Lanzhou lily. The extraction of total DNA from 12 soil samples was carried out using a soil DNA extraction kit (EZNA™ soil DNA Kit;

OMEGA Bio-tek, Inc., Doravilla, GA). Each sample was composed of 0.5 g fresh

soil. Extracted DNA was assayed for purity and concentration by ethidium bromide staining after 1.0% agarose gel electrophoresis, and the DNA was quantified by Ultra Micro-Spectrophotometer (NanoDrop 2000; Thermo Scientific, Wilmington, DE). Samples were centrifuged and diluted with sterile water to 1 ng/ μL for polymerase chain reaction (PCR) amplification. Fungal DNA was amplified by PCR (T100 gradient PCR; Bio-Rad, Hercules, CA), and the ITS1 (internal transcribed spacer 1) fragment was amplified. The primers used were specific primers ITS1F and ITS1R with Barcode, 5'-CTTGGTCATTTAGAGGAAGTAA-3' and 5'-GCTGCGTTCCTCATCGATGC-3'. The PCR reaction system was 30 μL , containing 15 μL of Phusion Master Mix (2 $\times$), 3 μL of primer (2 μM), 10 μL of DNA (1 ng/ μL), and 2 μL H_2O . PCR was performed using highly efficient and high-fidelity enzymes (Phusion® High-Fidelity PCR Master Mix with GC Buffer; New England Biolabs, Ipswich, MA) to ensure amplification efficiency and accuracy. The amplification procedure was as follows: 98 $^\circ\text{C}$ pre-denaturation 1 min; denaturation at 98 $^\circ\text{C}$ for 10 s; 50 $^\circ\text{C}$ annealing 30 s; 72 $^\circ\text{C}$ extension 30 s; cycle 30 times and then 72 $^\circ\text{C}$ extension 5 min. The PCR products were subjected to electrophoresis using 2% agarose gel. The PCR products were mixed at the same concentration, and after mixing thoroughly, purified by agarose gel electrophoresis with 2% of 1 $\times$ TAE and recycle target bands. The purification kit was supplied by Thermo Scientific's GeneJET Glue Recycling Kit. The library was constructed using the NEB Next® Ultra DNA Library Prep Kit for Illumina Library Kit of New England Biolabs. The constructed library was tested by Qubit quantification and examination. After passing, MiSeq was used for sequencing. There is a certain percentage of interference data in the raw data obtained by sequencing. To make the result of information analysis more accurate and reliable, the original data were spliced and filtered to obtain clean data. Based on valid data for operational taxonomic units (OTUs), data were clustered based on 97% similarity for species classification analysis. Combined OTU and species annotations were used to obtain the OTUs and classification profiling of each sample. After that, the abundance and diversity index of OTUs were analyzed. At the same time, the statistical analysis of community structure was carried out at each classification level.

Statistical analysis. The physiological indices of Lanzhou lily under PA stress were analyzed by EXCEL (Microsoft, Redmond, WA). Statistical analysis of fungal in continuous cropping rhizosphere soil: Application of IBM SPSS Statistics 19.0 (IBM Corp., Chicago, IL) one-way analysis of variance for the difference in the relative abundance of OTUs in soils of different continuous cropping years. Principal coordinate analysis uses QIIME for visualization.

Results

Effects of PA on plant organs of Lanzhou lily. With an increase of PA concentration, the root length, shoot height, and fresh weight of Lanzhou lily showed a decreasing trend, but the decrease was not significant. The root length of the lily was significantly decreased at 0.5 $\mu\text{mol}\cdot\text{g}^{-1}$ (Fig. 1A), and the fresh weight was significantly reduced at 0.25 $\mu\text{mol}\cdot\text{g}^{-1}$ (Fig. 1B), and the shoot height did not show significant difference between all treatments (Fig. 1C). The results show that the response of root growth to PA was more sensitive than that of the aboveground part in the plants. The effect of low concentration of PA was not significant, whereas high concentration had significant inhibitory effect on the growth of Lanzhou lily. The fresh weight of lily bulbs decreased with the increase of PA concentration and decreased significantly at 0.25 $\mu\text{mol}\cdot\text{g}^{-1}$ (Fig. 1D).

Effects of PA on activities of antioxidant protective enzymes in Lanzhou lily. With the increase of PA concentration, the activities of SOD, POD, and CAT in the roots of lily showed a tendency to increase first and then decrease. SOD and CAT activities reached the highest value at 0.01 $\mu\text{mol}\cdot\text{g}^{-1}$, and then decreased gradually at 1 $\mu\text{mol}\cdot\text{g}^{-1}$ and reached the lowest value, respectively, at 1.40 and 23.89 U/g (Fig. 2A and C). POD activity reached the highest value at 0.25 $\mu\text{mol}\cdot\text{g}^{-1}$, although the activity was decreased at 1.0 $\mu\text{mol}\cdot\text{g}^{-1}$, and the activity was still higher than that of the control (Fig. 2B).

Effects of PA on reactive oxygen and free radical scavenging ability of Lanzhou lily roots. The contents of H_2O_2 and MDA increased with the increase of PA concentration, and increased significantly at the concentration of 1 $\mu\text{mol}\cdot\text{g}^{-1}$, which was 37.8 $\mu\text{mol}\cdot\text{g}^{-1}$ and 0.6 nmol·g $^{-1}$, respectively (Fig. 3A and B). With the increase of the concentration of PA, the total phenolic content increased and later decreased, and it was significantly lower than that of the control at the concentration of 0.5 and 1 $\mu\text{mol}\cdot\text{g}^{-1}$ (Fig. 3C). The scavenging rate of hydroxyl radicals was not significantly changed at low concentrations and significantly decreased at high concentrations (no less than 0.5 $\mu\text{mol}\cdot\text{g}^{-1}$) (Fig. 3D).

Effects of PA on the root's vigor of lily. PA can decrease the root vigor of lily, and the decreased range increased with the increase of treatment concentration, while the concentration was 0.5 $\mu\text{mol}\cdot\text{g}^{-1}$, which was a significant decrease (Fig. 4). This indicated that the inhibitory intensity of lily root activity was not significant while the PA concentration was below 0.5 $\mu\text{mol}\cdot\text{g}^{-1}$, and the inhibitory effect was enhanced with the increase in concentration.

Effects of PA on cytochrome of lily leaves. Chlorophyll A, chlorophyll B, and carotenoid content decreased with the increase of PA concentration, whereas the decrease was not significant at low concentration, and significant at high concentration. Chlorophyll A, chlorophyll B, and carotenoid contents were significantly lower at 0.5 and 1 $\mu\text{mol}\cdot\text{g}^{-1}$

Fig. 1. Effect of phthalic acid (PA) on root length (A), fresh weight (B), shoot height (C), and bulb fresh weight (D) of Lanzhou lily plant. Data are the means of three replicates. Different letters above error bars indicate significant differences at the 5% level by the Duncan test.

Fig. 2. Effects of phthalic acid (PA) on enzyme activities of superoxide dismutase (SOD) (A), peroxidase (POD) (B), and catalase (CAT) (C) of Lanzhou lily roots. Data are the means of three replicates. Different letters above error bars indicate significant differences at the 5% level by the Duncan test. FW = fresh weight.

(Fig. 5). This indicated that the low concentration of PA had little effect on the chlorophyll and carotenoid content of lily leaves,

whereas the content of chlorophyll and carotenoid was significantly decreased at high concentration.

Sequencing data and fungi profile. The original tags were split, stitched, intercepted, and filtered, resulting in a total of 621083 valid tags, whereas the number of tags used to build OTUs and obtaining the classified information was 615030. The clustering of the tags obtained 855 OTUs based on 97% similarity (Fig. 6). The number of fungi sequences obtained from RS0, RS1, RS2, and RS3 rhizosphere soil samples were $46,779 \pm 16,184$, $44,314 \pm 14,782$, $55,120 \pm 5222$, and $58,796 \pm 1327$ (mean $\pm$ SD), respectively. The sequence length ranged from 213 to 368 base pairs. The results of sparse curves show that the abundance of OTU (abundance of species) increases with the number of extracted sequences, and the curve tends to increase and reaches an equilibrium state when reaching 25,000 (Fig. 7). Fungi OTUs consisted of five phyla, with Ascomycota (65.1% of the total abundance), followed by Zygomycota (12.3%), Basidiomycota (3.5%), Chytridiomycota (0.09%), and Glomeromycota (0.01%) (Fig. 8).

Effects of continuous cropping of Lanzhou lily on the diversity and structure of the fungal community. To determine the change of fungal community diversity, the OTU abundance, Chao1 index, and Shannon index were selected as the diversity index. At the species level, continuous cropping of Lanzhou lily had a significant effect on OTU abundance and Shannon index. At the genus level, continuous lily had no significant effect on OTUs and Chao1, but had a significant effect on the Shannon index (Table 1). Continuous cropping for 2 years (RS2) significantly reduced the Shannon index, whereas for 3 consecutive years (RS3) slightly reduced the Shannon index (Table 1).

The fungal community structure of four different lily fields was compared at the class level (Table 2). Continuous planting lily for 3 years (RS3) significantly increased the relative abundance of Sordariomycetes, Eurotiomycetes, Dothideomycetes, and Pezizomycetes, and 1 and 2 years (RS1 and RS2) did not significantly affect the relative abundance of Sordariomycetes, Eurotiomycetes, and Dothideomycetes (Table 2). RS3 significantly reduced the relative abundance of Agaricomycetes and RS1 significantly increased the relative abundance of Agaricomycetes. Compared with the control fields of no lily cropping (RS0), RS1, RS2, and RS3 had no significant effect on Chytridiomycetes, Tremellomycetes, Leotiomycetes, and others (Table 2).

Effects of continuous cropping of Lanzhou lily on relative abundance of specific fungi. The 35 OTUs with the longest sequence number in all soil samples was 78.5% of the total number of sequences, and the first 5 OTUs were *Plectosphaerella cucumerina*, *F. oxysporum*, *Mortierella alpina*, uncultured zygomycete, and *Stilbella aciculosa*. The relative abundance of *F. oxysporum*, *Penicillium* sp., *Alternaria longissima*, *Botrytis cinerea*, and *Colletotrichum circinans* showed an increasing trend with the lengthening of the years of lily continuous cropping, and all

Fig. 3. Effects of different concentration of phthalic acid (PA) on H₂O₂ (A), Malondialdehyde (MDA) (B), total phenolics (C), and scavenging rate of hydroxyl radical (D) of Lanzhou lily roots. Data are the means of three replicates. Different letters above error bars indicate significant differences at the 5% level by the Duncan test. FW = fresh weight.

Fig. 4. Effects of phthalic acid (PA) on root vigor of Lanzhou lily. Data are the means of three replicates. Different letters above error bars indicate significant differences at the 5% level by the Duncan test.

of them increased significantly when the lily was continuous cropping for 3 years. *F. oxysporum* had a relatively high abundance, reaching $12.3\% \pm 0.2\%$ (mean $\pm$ SE) at 3 years, whereas *C. circinans* had a relatively low abundance, $0.007\% \pm 0.001\%$. The relative abundance of *Fusarium solani*, *Fusarium equiseti*, and *Ilyonectria macrodidyma* showed a decreasing trend with the extension of the continuous cropping period. Lily continuous cropping for 2 years (RS2) significantly reduced the relative abundance of *F. solani* and *F. equiseti*, whereas the decrease in *I. macrodidyma* was not significant (Fig. 9).

Lily continuous cropping variously reduced the relative abundance of *M. alpina*, Glomeromycota, *Trichoderma* sp., and *M. alpina* and Glomeromycota showed a significant decrease in lily continuous cropping for 2 years (RS2) and 3 years (RS3). The relative abundance of *M. alpina* reduced from $9.9\% \pm 1.8\%$ to $4.7\% \pm 0.9\%$. Glomeromycota and *Trichoderma* sp. have a lower relative abundance, and Glomeromycota was only 0.003% at 2 years, *Trichoderma* sp. was not found in soil samples for lily continuous cropping 1 year (Fig. 10).

Discussion

PA significantly inhibited the growth of lily plants by reducing root length, root activity, and leaf photosynthesis, leading to a decrease in lily bulb production (Fig. 1D). The stem root system significantly effects the growth and development of lily shoots (Song, 2017). Meanwhile PA induced oxidative stress and lipid peroxidation of lily roots, increased the content of H₂O₂ and MDA, and induced the changes of activities of antioxygen protective enzymes and the content of total phenol. Other research demonstrates the phytotoxicity of PA, which inhibited the growth of apple plants by inducing oxidative stress and oxidative damage (Bai et al., 2009).

Reactive oxygen species (O₂^{•-}, H₂O₂, •OH) and their associated oxidative stress are considered to be an action mechanism for

allelochemicals to inhibit plants (Weir et al., 2004). The allelochemicals are mainly formed by the formation of semiquinone free radicals, transferring electrons to molecular oxygen to form superoxide radicals (O₂^{•-}), and then through a series of reactions to become more active hydroxyl radicals (•OH) or oxidation of hydroxyl radicals (HO₂[•]) (Hammond-Kosack and Jones, 1996). Hydroxyl radicals are the most active free radicals (Beckman et al., 1990), which act on biological macromolecules, such as proteins, nucleic acids, and lipids, resulting in damage to cell structure and function, leading to metabolic disorders that cause disease. Therefore, the hydroxyl radical scavenging ability is one of the important indicators of antioxidant capacity in plants (Fox, 1984; Husain et al., 1987; Smirnov and Cumbes, 1989). H₂O₂ is the most common reactive oxygen molecule (Apel and Hirt, 2004), and as a signal molecule mediates a series of biochemical reactions during plant growth (Deng et al., 2012). MDA is one of the membrane peroxidation products after free radical initiated, often as an indicator of the degree of membrane lipid (Vilaplana et al., 2006). Our results showed that PA increased the contents of H₂O₂ and MDA in Lanzhou lily roots, indicating that PA induced root oxidative stress, and then damaged the cell membrane structure. With the increase of PA concentration, the contents of H₂O₂ and MDA increased (Fig. 3A and B), which indicated that the remaining active oxygen in the lily roots increased, and the membrane lipids were damaged by excessive free radicals, thereby elevating levels of membrane lipid peroxide. The scavenging ability of hydroxyl radicals decreased with the increase of PA concentration, which exacerbated injury of free radicals to cells with the increased concentration.

Continuous accumulation of reactive oxygen species under stress can activate plant cell defense genes (Dayan and Watson, 2011), thus the activity of antioxygen protective enzymes to remove reactive oxygen species, for example, SOD, POD, and CAT, ascorbate peroxidase, glutathione reductase, and glutathione peroxidase (Blokina et al., 2003; Nakano and Asada, 1981). SOD as the main superoxide (•O₂⁻) scavenger, which converts the superoxide (•O₂⁻) into H₂O₂ and O₂, plays an important role in defense of cell damage (Hasan et al., 2009; Meloni et al., 2003). With the increase of PA concentration, the activities of SOD, POD, and CAT in the roots of lily tended to increase first and then decrease (Fig. 2). The results show that low concentrations of PA could promote the protective effect of root on its own. With an increase in the concentration of PA, the oxidative stress on the lily plant was deepened and the activity of SOD, POD, and CAT decreased. Zhang et al. (2014) studied effects of di-n-butyl phthalate on cucumber and had similar findings (Zhang et al., 2014). Jiang and Yang (2009) also found similar trends in the effects of different concentrations of herbicide on the antioxidant enzyme

activity of wheat. Phenolics, as nonenzymatic antioxidants, can scavenge free radicals and resist oxidation (Chimi et al., 1991; Dinis et al., 1994; Sakihama et al., 2002). In

our research, as the concentration of PA increased, the total phenolic content also showed a tendency to increase first and then decrease (Fig. 3C). This indicates that the

increase of oxygen free radicals leads to an increase of the total phenolic content to remove oxygen free radicals in the low-concentration treatment, whereas excess oxygen free radicals at high concentrations may affect the metabolism of the cells, resulting in a decrease in the total phenolic content.

Root vigor can be used as an indicator of root absorptency and metabolism, and can reflect plant growth ability to a certain extent (Li et al., 2011; Liu et al., 2014). In this study, PA decreased the activity of lily roots. This indicated that PA could inhibit the absorption of water and mineral elements in the soil by affecting the root activity, which in turn affected the normal growth of the Lanzhou lily.

Chloroplast photosynthetic pigments are an important basis for photosynthesis of plants, and their levels reflect the potential of plant photosynthesis to a certain extent (Ahmed et al., 2012). Carotene is an oxygen free radical scavenger on the chloroplast membrane that is associated with photosynthetic centers PSI and PSII to protect chlorophyll from oxidation and reduce free radicals (Demmig-Adams and Adams, 1996; Farquhar and Sharkey, 1982; Reddy et al., 2004). Chlorophyll A, chlorophyll B, and carotenoids decreased with the increase of PA concentration, whereas the decrease was not significant at low concentration (Fig. 5). This indicates that the low concentration of PA is less damaging and does not cause a decrease in the total chlorophyll content. When the content of carotenoids decreased at high-concentration PA, it was difficult to remove excess oxygen free radicals in time. The chlorophyll was oxidized and content

Fig. 5. Effects of phthalic acid (PA) on the content of chlorophyll A (A), chlorophyll B (B), and carotenoid (C) of Lanzhou lily leaves. Data are the means of three replicates. Different letters above error bars indicate significant differences at the 5% level by the Duncan test. FW = fresh weight.

Fig. 6. Number of Tags and operational taxonomic units (OTUs) recovered from various soil samples. RS0.1, RS0.2, and RS0.3 indicate three replicates in RS0; other treatments include the similar replicates. RS0 = control field of no lily cropping; RS1 = field of 1-year, RS2 = field of 2-year, RS3 = field of 3-year consecutive lily cropping.

decreased significantly, which resulted in the decrease of photosynthetic rate and the decrease of photosynthetic products. This result is consistent with the inhibitory effect of other allelochemicals on plant photosynthesis (Han et al., 2009; Jaleel et al., 2008; Pan et al., 2011; Pinto et al., 2003; Prasad and Zeeshan, 2005).

Continuous cropping is often influenced by autotoxicity. Our study showed that accumulation of PA in rhizosphere soil as under continuous cropping of Lanzhou lily could cause oxidative damage of Lanzhou lily roots and inhibit the growth of the lily. Phthalate esters are the major autotoxic agents of tobacco root exudates that affect seed germination and seedling growth and cause tobacco autotoxicity (Deng et al., 2017).

The change of the fungal community in rhizosphere soil samples from plots with different lily cropping duration was determined by pyrosequencing. The results showed that the diversity of fungal communities decreased trend with an increase in continuous cropping duration. The composition and structure of fungal communities had significant differences among different soil samples.

The diversity of fungal communities in continuous cropping potato plots was significantly lower than that of rotation plots (Manici and Caputo, 2009). In our research, the fungal OTUs contained five phyla, the main is Ascomycota, followed by Zygomycota and Basidiomycota (Fig. 8). This result is consistent with the results of Xingang Zhou and Fengzhi Wu (Zhou and Wu, 2012). They added coumaric acid to the cucumber continuous cropping soil to simulate the change of microbial community. The results showed that the main fungi were Ascomycota and Zygomycota at the phylum level. In our study, the relative abundance of Chytridiomycota (0.09%) and Glomeromycota (0.01%) was the lowest, consistent with the results of Xu et al. (2012), who found that the relative abundance of Chytridiomycota and Glomeromycota was the lowest in all strains, 0.1% and 0.03%, respectively (Xu et al., 2012). At the level of class, Sordariomycetes was the dominant species, and the relative abundance of fungi in the intercropping soil was significantly higher than that in the control. The Dothideomycetes significantly increased in the intercropping for 2 and 3 years, whereas the Agaricomycetes were significantly reduced in the intercropping for 3 years, indicating that the long-term continuous cropping increased Sordariomycetes and Dothideomycetes, and reduced the relative abundance of Agaricomycetes. These results are consistent with those reported by Li et al. (2014). Earlier studies have found that Lanzhou lily root rot is mainly caused by *Fusarium* spp., including *F. oxysporum*, *F. moniliforme*, and *F. salani*; *F. oxysporum* has the highest separation frequency and pathogenicity as the main pathogen (Shang et al., 2014). In this study, except for *P. cucumerina*, the highest absolute abundance of OTU was *F. oxysporum*, and its relative abundance showed an increasing trend with the extension of the continuous cropping period, and the RS3 significantly higher than that of the RS0, which is 12.3%. In summary, *F. oxysporum*

Fig. 7. Rarefaction curves show the relationship between sampling intensity and the number of recovered operational taxonomic units from soil of lily fields. RS0.1, RS0.2, and RS0.3 indicate three replicates in RS0; other treatments include the similar replicates. RS0 = control field of no lily cropping; RS1 = field of 1-year, RS2 = field of 2-year, RS3 = field of 3-year consecutive lily cropping.

Fig. 8. Relative abundances of the main fungal phylum in various soil sample. RS0.1, RS0.2, and RS0.3 indicate three replicates in RS0; other treatments include the similar replicates. RS0 = control field of no lily cropping; RS1 = field of 1-year, RS2 = field of 2-year, RS3 = field of 3-year consecutive lily cropping.

Table 1. Diversity indexes of fungal communities.

Soil sample	OTU (species)	OTU (genus)	Chao1 (species)	Chao1 (genus)	Shannon (species)	Shannon (genus)
RS0	424.7 ± 16.8 a	370.0 ± 12.2 a	509.6 ± 51.9 a	416.3 ± 32.6 a	6.0 ± 0.0 a	5.8 ± 0.0 a
RS1	412.0 ± 30.8 ab	363.7 ± 31.5 a	492.2 ± 47.3 a	434.0 ± 51.1 a	5.5 ± 0.1 ab	5.3 ± 0.1 ab
RS2	325.7 ± 33.7 b	286.3 ± 28.7 a	404.3 ± 29.5 a	349.7 ± 32.5 a	4.6 ± 0.6 b	4.5 ± 0.6 b
RS3	418.7 ± 23.3 a	369.3 ± 22.3 a	516.1 ± 32.1 a	432.1 ± 24.2 a	5.7 ± 0.1 ab	5.4 ± 0.1 ab

Data are means of three replicates with SE. Letters within a column indicate significant differences at the 5% level by the Duncan's multiple range test. OTU = operational taxonomic units; RS0 = control field of no lily cropping; RS1 = field of 1-year, RS2 = field of 2-year, RS3 = field of 3-year consecutive lily cropping.

Table 2. Relative abundances of the main fungal classes in various soil samples.

Fungal classes	RS0	RS1	RS2	RS3
Sordariomycetes	46.77 ± 2.27 a	43.37 ± 3.65 a	47.50 ± 1.30 a	56.82 ± 0.96 b
Agaricomycetes	0.84 ± 0.18 b	1.69 ± 0.16 a	1.18 ± 0.13 b	0.29 ± 0.06 c
Eurotiomycetes	1.45 ± 0.36 b	1.31 ± 0.31 b	1.91 ± 0.21 ab	2.76 ± 0.35 a
Dothideomycetes	0.91 ± 0.08 bc	0.74 ± 0.04 c	1.26 ± 0.17 b	1.80 ± 0.10 a
Chytridiomycetes	0.06 ± 0.01 a	0.10 ± 0.07 a	0.14 ± 0.07 a	0.06 ± 0.02 a
Tremellomycetes	1.10 ± 0.23 a	0.73 ± 0.12 a	0.65 ± 0.25 a	0.95 ± 0.13 a
Pezizomycetes	0.60 ± 0.15 b	0.30 ± 0.05 b	1.02 ± 0.08 a	1.18 ± 0.08 a
Leotiomycetes	0.73 ± 0.14 a	0.67 ± 0.18 a	0.92 ± 0.28 a	0.81 ± 0.32 a
Others	0.11 ± 0.02 a	0.13 ± 0.01 a	0.10 ± 0.01 a	0.09 ± 0.01 a

Data are means of three replicates with se. Letters within a column indicate significant differences at the 5% level by the Duncan's multiple range test.

RS0 = control field of no lily cropping; RS1 = field of 1-year, RS2 = field of 2-year, RS3 = field of 3-year consecutive lily cropping.

Fig. 9. Relative abundances of eight groups of fungi: *Fusarium oxysporum*, *Fusarium solani*, *Fusarium equiseti*, *Alternaria longissima*, *Ilyonectria macrodidyma*, *Botrytis cinerea*, *Colletotrichum circinans*, and *Penicillium sp.* in all of the soil samples. Data are means of three replicates. Letters above error bars indicate significant differences at the 5% level by the Duncan multiple range test. RS0 = control field of no lily cropping; RS1 = field of 1-year, RS2 = field of 2-year, RS3 = field of 3-year consecutive lily cropping.

Fig. 10. Relative abundances of eight groups of fungi, *Mortierella alpina*, uncultured Basidiomycota, Glomeromycota, and *Trichoderma* sp. in all of the soil samples. Data are means of three replicates. Letters above error bars indicate significant differences at the 5% level by the Duncan multiple range test. RS0 = control field of no lily cropping; RS1 = field of 1-year, RS2 = field of 2-year, RS3 = field of 3-year consecutive lily cropping.

may be the main pathogen in the Lanzhou lily of this study, and the increase in relative abundance exacerbates the disease. In this study, we found *Penicillium* sp., *A. longissim*, *B. cinerea*, and *C. circinans*, and their relative abundance increased with the extension of the continuous cropping period, and increased significantly in plots of continuous cropping for 3 years. Meanwhile, these fungi were reported to cause plant disease (Kiehr et al., 2012; Lou et al., 2013; Thomma, 2003). Therefore, *Penicillium* sp., *A. longissima*, *B. cinerea*, and *C. circinans* are likely to be the pathogenic fungi of Lanzhou lily. This study also found *I. macrodidyma*, which has been reported to be an olive tree root rot pathogen (Urbez-Torres et al., 2012). In addition, the study found that the relative abundance of *M. alpina*, Glomeromycotan fungus, and *Trichoderma* sp. within the lily continuous cropping showed a downward trend. Glomeromycota (AMF) and *Trichoderma* sp. are plant-beneficial bacteria that can improve plant resistance (Kubicek et al., 2001; Whipps, 2004). The reduction of these beneficial bacteria may be due to the accumulation of carbon deposited in the rhizosphere soil of Lanzhou lily. The increase of the fungal pathogen population also exacerbates the competition with beneficial bacteria. The reduction of these beneficial bacteria reduces the inhibition in soil-borne pathogens and competition for resources, potentially exacerbating the occurrence of disease in Lanzhou lily.

Conclusion

In conclusion, high concentrations of PA resulted in inhibited the growth of Lanzhou lily, which may be one of the mechanisms that trigger continuous cropping obstacles of

Lanzhou lily. With the increase of continuous cropping time, the diversity of fungal communities showed a decreasing trend. The composition and structure of fungal communities had significant differences among different soil samples. *F. oxysporum* may be the major pathogen in the Lanzhou lily of this study, and the increase in relative abundance exacerbates the decrease in yield and occurrence of disease. Lanzhou lily continuous cropping resulted in an increase in fungal pathogens, and reduced the size of the beneficial bacteria, which may be the main cause of increase in disease of Lanzhou lily.

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