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Dibutyl phthalate contamination remolded the fungal community in agro-environmental system

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HIGHLIGHTS

- DBP would change both α and β diversities of fungal community.
- The variation of fungal community were influenced by DBP pollution, bacterial community and soil properties.
- The molecular ecological network structure of fungal community was destabilized under DBP stress.

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ABSTRACT

Dibutyl phthalate (DBP) is a typical soil contaminant that is widely used as plasticizer in modern agricultural production. In this study, an experiment was conducted to evaluate fungal community succession in a soil-vegetable ecosystem under different DBP concentrations. By using high-throughput sequencing of the ribosomal internal transcribed spacer (ITS) region, it was shown that DBP contamination caused significant changes to the soil fungal community, in terms of both α and β diversities. The largest changes in fungal α and β diversities were detected under 50 mg/kg DBP concentration at the first day of addition. The bulk soils, rhizosphere soils and the phyllosphere harbored different fungal communities, while the abundance of saprotrophs and plant pathogens in the phyllosphere have been increased under DBP contamination. From correlation analysis and partial Mantel test, the change in fungal community α diversity was the result of multiple factors (DBP concentration, bacterial community and soil properties) while the β diversity of fungal community was mainly co-varied with the bacterial community after DBP contamination. Moreover, molecular ecological network analysis demonstrated that DBP contamination was detrimental to mutualistic relationships among fungal species and destabilized the network structure. Overall, the fungal communities in soils and around vegetables were largely remolded by DBP contamination that provides new insight into DBP contamination impacts on agricultural ecosystems.

1. Introduction

The application of greenhouses and mulching films in cold season agricultural production plays an important role in increasing the supply of fresh produce, resulting in social and economic benefits. However, the phthalate esters (PAEs) in the

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plastic components utilized in those structures may cause a health risk to people who eat these agricultural products (Fang et al., 2017; Lin et al., 2017). PAEs are one group of endocrine-disrupting chemicals and are often used as plasticizers (Cai et al., 2017). In China, about 90% of the polyvinyl chloride produced contain PAEs (He et al., 2015). As a representative PAE, DBP is easily released to the surrounding environment because they are not chemically bonded to plastic (He et al., 2015). Many researches have investigated the DBP concentration in agricultural soils. Zhang et al. (2013) found that DBP concentration in typical plastic mulched crops soils in Qingdao (China) was 9.02–13.01 mg/kg and it was 110 times higher than allowable concentration. Li et al. (2016) found that the DBP concentration in greenhouse vegetable production bases (Beijing, China) was within the scope of 0.44–1.23 mg/kg. Wang et al. (2015a) investigate the PAEs concentration in suburban plastic film greenhouses (Nanjing, China) and found that DBP concentration was range from ND-2.08 mg/kg. DBP in agricultural environments can be taken up by crops, thereby entering into the food chain and eventually reaching the human body (He et al., 2013). The Environmental Monitoring Center of China and the United States Environmental Protection Agency have both listed DBP as a priority pollutant due to its carcinogenicity, teratogenicity and mutagenicity (Jin et al., 2015). Also, many studies have demonstrated that DBP can accumulated in plants (Li et al., 2016; Wang et al., 2015a) and affect the growth of crops, including cucumber (Wang et al., 2016a; Zhang et al., 2014, 2015a, 2015b), pumpkin (Lin et al., 2017), Chinese flowering cabbage (Zhao et al., 2016), wheat (Gao et al., 2016) and rice (Cai et al., 2017). Consequently, the risks of DBP contamination in agricultural ecosystems have attracted a great deal of public attention. But how DBP affects the functioning of ecosystem remains unclear. Some studies have focused on the bacterial community responses to the DBP contamination, in soils and within plants (Kong et al., 2018; Xie et al., 2010; Zhang et al., 2015b). However, as yet no study has explored the changes in the fungal community after DBP pollution.

Fungi are an important component of the terrestrial ecosystem and play crucial roles in a wide range of biogeochemical processes, including decomposition, soil nutrient mobilization and conversion, and plant nutrition absorption (Han et al., 2016; Schimann et al., 2017; Tu et al., 2015). The fungal community can also affect plant growth via mutualistic or pathogenic interactions (Hiruma et al., 2018; Raman and Suryanarayanan, 2017). A number of factors can affect their community as well, such as long-term phosphorus fertilization (Beauregard et al., 2010), land-use types (Lauber et al., 2008), soil pH (Rousk et al., 2010), geographic distance (Talbot et al., 2014), forest age (Han et al., 2016), desertification (Wang et al., 2016b), and soil contamination (Wang et al., 2016c). However, succession in the fungal community in polluted soil-vegetable ecosystems has not been studied. Knowledge of how fungal communities respond to DBP contamination is critical for bioremediation measures and health risk assessments. In addition, the phyllosphere microbial community was important to many global process and their host plant (Lindow and Brandl, 2003). So the effect of DBP contamination to phyllosphere fungal community is required to be investigated.

Here, a pot experiment was used to simulate an agricultural ecosystem with vegetable growth. *Brassica napus* L. (a widely eaten leafy green in China) was used as a model vegetable in this study. Environmental DNA from bulk soil, rhizosphere soil and phyllosphere samples were collected and the fungal phylogenetic marker (ribosomal internal transcribed spacer, ITS) was sequenced by high-throughput sequencing technology. The main objectives of this study were to (i) monitor the variation of fungal communities in soils and phyllosphere of *Brassica campestris* L. after DBP pollution, and (ii) better understand the main environmental factor that could alter the fungal community during the soil pollution. This research greatly increases what is known about the effects of DBP on fungal community in common soil-vegetable ecosystem.

2. Materials and methods

2.1. Pot experiment and sampling

The experimental soil was collected from surface layers (0–20 cm) at Huairou District, Beijing, China (40°16′42″N, 116°20′07″E). The original soil properties are as follows, pH 7.73, total phosphorus (TP) 1923 mg/kg, total nitrogen (TN) 834 mg/kg, total soil organic carbon (SOC) 0.84%, and an initial DBP concentration of 1.71 mg/kg. The soil was air-dried, ground, and sieved through 20 mesh. The vegetable seeds were purchase from ZSDSL Flower Market Co., Ltd. (Beijing, China) and sprouted before sowed. At the beginning, the seeds were surface sterilized with 70% ethanol (1 min) and 2.5% sodium hypochlorite (5 min). After the disinfectant were washed off completely, the seeds were soaked in warm water (50-55 °C) for 20 min and soaked in water at room temperature (25 ± 3 °C) for 3 h, then incubated at low-temperature environment until germination.

The experiments were conducted in a greenhouse at the Research Center for Eco-Environment Sciences, CAS (40°01′47″N, 116°35′07″E, Beijing, China) from June 25, 2016 to July 25, 2016. Four treatments with different levels of DBP (≥98%, Alfa Aesar, USA) concentration were set up: CK (0 mg/kg), C1 (50 mg/kg), C2 (200 mg/kg), C3 (500 mg/kg). A certain amount of mother solution of DBP (10 g DBP/L *n*-hexane) were added to soil and mixed thoroughly. After *n*-hexane volatilization, total 2.5 kg of the soil were weighed and placed in each ceramic pots (20 cm in height × 18 cm in diameter at top and 13 cm diameter at base). All treatments were set up in 6 replicates. The soil samples were collected at day 1, 3, 5, 10, 20, and 30 (soil collected at day 30 included both bulk soil and rhizosphere soil (R)). The vegetable roots were wash 3 times with PBS buffer after bulk soils were shaking off from the roots. The suspensions were pooled and centrifuged, the resulting sediment pellets was identified as rhizosphere soils. Plants were collected on day 30, and the phyllosphere microbial communities (P) were collected as previously reported (Delmotte et al., 2009; Xie et al., 2015). Briefly, the leaves were submerged in 100 ml of PBS (0.02 M, pH 7.0 and contain 0.1% Tween 80), and sonicated for 10 min at 20000 HZ in an ultrasonic cleaner water bath (Ningbo Xingzhi Biological Technology Co. Ltd., Ningbo, China). The microorganisms collected by vacuum filtration and the obtained Millipore filtration (0.22 μm, Billerica, MA, United States) were preserved in −80 °C before DNA extraction.

2.2. DNA extraction, PCR amplification and high-throughput sequencing

The genomic DNA were extracted using Fast DNA spin kit for soil (MP Biomedicals LLC, USA) according to the manufacturer's instructions. The ITS region was amplified with primer gITS7 (5′-GTGARTCATCGARTCTTTG-3′) (Ihrmark et al., 2012) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Primers contained unique barcodes to enable sequencing of multiple samples in a single sequencing run. The PCR was performed in a 50 μL mixture which included 5 μL of 10 × PCR buffer, 4 μL of dNTPs, 0.5 μL of Taq DNA polymerase (TaKaRa Biotech, Beijing, China), 1.5 μL of 10 μM each primer, 20–30 ng of genomic DNA, and water up to 50 μL. The thermal cycling conditions were as follows: 94 °C for 1 min, 35 cycles of 94 °C for 20 s, 57 °C for 25 s, 72 °C for 45 s, with an extension at 72 °C for 10 min. The PCR products were visualized on a 1% agarose gel and the target bands were extracted