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Research paper

Loss of soil microbial diversity may increase insecticide uptake by crop

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ABSTRACT

Belowground biodiversity is essential for soil functioning, but the effect of belowground biodiversity loss on food safety is unknown. We investigated the loss of soil microbial diversity on insecticides accumulation in Brassica. We manipulated soil biodiversity using the dilution-to-extinction approach in a Brassica-soil-insecticide system, monitored microbial communities via high-throughput sequencing, and identified potential functional microbes. Compared with unsterilized soil, the richness of functional bacteria was reduced by 14.1%, 36.2%, 51.6% and 73.2%, respectively, in the corresponding sterilized soil inoculated with 1-, 10^{-2}-, 10^{-4}- and 10^{-6}-fold diluted soil suspension. The acetamiprid and imidacloprid concentrations increased significantly in Brassica tissues grown in sterilized soil inoculated with 10^{-6}-fold diluted suspension. A bacterial group predominated in functional microbes of soils inoculated with 1-, 10^{-2}- or 10^{-4}-fold diluted suspension, but the relative abundance declined in soil inoculated with a 10^{-6}-fold diluted suspension. Our findings revealed that undesirable impacts by the loss of soil biodiversity at an intermediate level on the accumulation of soil contaminant in plants could be alleviated by microbial functional redundancy through disproportionally complementary growth of specific functional microbial taxa, but severe loss of soil biodiversity would threaten food safety.

1. Introduction

Biodiversity is greatly affected by human activity, such as changes in land-use and increase in nitrogen deposition (Sala et al., 2000; Butchart et al., 2010). The relationship between biodiversity and ecosystem functioning has become one of the research focuses in the last three decades (Loreau et al., 2001; Cardinale et al., 2012). It has been shown that reduction in biodiversity results in decreases in the productivity, functionality, and stability of ecosystems (Loreau et al., 2001; Cardinale et al., 2012). However, previous studies have mainly focused on aboveground ecosystems. The relationship between belowground diversity and ecosystem functioning has remained less investigated even though biodiversity is usually richer in belowground systems than in aboveground systems (Bardgett and van der Putten, 2014).

Previous reports showed effects of reduction in soil biodiversity on soil functions were controversial. It has been hypothesized that the loss of belowground biodiversity would be less important than the loss of aboveground diversity in ecosystem functioning because belowground species are mainly microbes with high diversity and high functional redundancy (Nannipieri et al., 2003). Several empirical studies have indeed shown consistency with this hypothesis. For instance, reduction in soil biodiversity does not necessarily result in reduction in the efficiency of straw decomposition, transformation of soil organic carbon and denitrification. However, some other functions may even increase when soil biodiversity reduces (Griffiths et al., 2001; Wertz et al., 2006). It appears that whether biodiversity affects functions of ecosystems depends on the types of processes tested. The processes carried out by a large group of highly diverse microbes are usually less affected, whereas the processes carried out by less diverse

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microbes are more seriously affected when specific microbes decline (Peter et al., 2011; Prosser, 2012; Hernandez-Raquet et al., 2013; Philippot et al., 2013). One of the reasons leading to the controversy in the function-diversity relationship is that diversity of total microbiome rather than functional microbes involved in the targeted function was examined. How the functional microbes respond to a decline in soil overall biodiversity is not well understood.

Emphases of previous studies on the ecological processes mediated by soil biodiversity are placed on those essential ecosystem functioning. The ecological processes related directly to human health have rarely been explored (Hernandez-Raquet et al., 2013; Wall et al., 2015). Utilization of insecticides for pest control is a common practice in modern agriculture. Most of the applied insecticide residues enter into the soil and a portion of them get into harvested food. Thus the accumulation of insecticides in soil would affect the safety of environments and food, and consequently the health of humans. It is known that intensive agricultural managements reduce soil biodiversity (Tsiafouli et al., 2015; Xun et al., 2015). However, it is not known if and how a reduction in soil biodiversity affects the degradation of insecticides in soil and the uptake of insecticides by crops. The objective of this study is to test the hypothesis that loss in diversity of functional microbes inhibits insecticide degradation in soil and results in higher uptake of insecticides by crops. To test this hypothesis, we monitored the diversity of functional microbes relating to degradation and crop uptake of insecticide with high-throughput sequencing by comparing relative abundance of each operational taxonomic unit (OTU) between treatment and control samples.

2. Materials and methods

2.1. Soil collection and treatments

Top soil (0–20 cm) was collected from a vegetable garden in the Institute of Botany, the Chinese Academy of Sciences, Beijing, China, where no insecticides investigated in the present study were applied in the previous five years. The soil is classified as Drab Fluvo-aquic type, with pH 7.61, soil organic carbon 11.55 g kg^{-1}, total nitrogen 0.68 g kg^{-1}, total phosphorus 0.57 g kg^{-1}, nitrate 16.71 mg kg^{-1}, ammonium 6.99 mg kg^{-1}, Olsen phosphorus 42.78 mg kg^{-1}, and available potassium 65.64 mg kg^{-1}. After sieving through 3 mm mesh to remove stone and vegetable residues, the soil was distributed to plastic bags (2 kg each). Then all the bags were closed by sterilized cotton stopper and sterilized with 30 kGy of gamma radiation at Institute of Food Science and Technology, Chinese Academy of Agricultural Science.

The experiment was carried out in a two-way random block design. One factor was soil microbial dilution at five levels, i.e. unsterilized soil (defined as NS, hereafter), sterilized soils inoculated with the undiluted soil suspension (defined as 1-suspension hereafter), and soil suspensions diluted for 10^{-2}, 10^{-4}, and 10^{-6} fold (defined as 10^{-2}-, 10^{-4}-, and 10^{-6}-suspension, respectively, hereafter). The other factor was insecticide treatments at two levels, i.e. no insecticide addition as control (designated as CK) and insecticide addition (Insecticide). Each treatment was replicated five times.

2.2. Soil suspensions preparation and inoculation

The 1-suspension was made by adding 100 g unsterilized soil (which was the same soil used for sterilization) to 1000 mL autoclaved distilled water and stirred for 10 min. Ten milliliter of the 1-suspension was added to 990 mL aseptic water to get 10^{-2}-suspension. The 10^{-4}- and 10^{-6}-suspensions were made in the same way. Sterilized soil in different bags was inoculated with 50 mL of the dilution series of the soil suspension in aseptic hood, re-closed by sterilized cotton stopper and incubated at 25 °C in the dark for 8 weeks to allow microbial regeneration. The final moisture was adjusted to 60% water holding capacity of the soil with autoclaved water.

2.3. Insecticide addition

Acetamiprid, chlorpyrifos and imidacloprid were selected in the present study because they are widely used in agriculture. Before sowing, an insecticide mixture with equal amounts of acetamiprid, chlorpyrifos and imidacloprid were first dissolved as an emulsifiable concentrate and then diluted with sterilized water. Each insecticide was added to a final concentration at 30 μg g^{-1} soil, remained static overnight and homogenized, and then the bags were transferred to plastic pots. CK treatment received the same volume of oil concentrate but without insecticides in sterilized water. The dose of insecticide addition was based on the recommended rates for soil borne insecticide control (Baskaran et al., 1999; Serikawa et al., 2012). We used an insecticide mixture to increase the possibility of detection.

2.4. Planting and sample collection

Ten surface-sterilized Brassica (*Brassica chinensis* L.) seeds were sown to each pot and five plants with uniform size were kept one week later. Pots were placed in greenhouse and subjected to regular exchange of locations once a week. Plants were irrigated with autoclaved water and received no pest or disease control during the experiment. Half-lives of these three insecticides degradation vary widely in different soils, with average around 15 d (Singh et al., 2003; Liu et al., 2006; Gupta and Gajbhiye, 2007). Thus small portions of soil were taken from each pot at 15 d after sowing to monitor insecticide degradation in soil. The soil samples were stored at −20 °C for insecticide assays. Brassica plants were harvested at 60 d. After measuring fresh weights, half of the Brassica tissues were stored at −20 °C for insecticide analysis and the rest were oven-dried at 70 °C for 72 h to measure dry weight. Soils were homogenized and stored at 4 °C for nutrients determination and at −20 °C for molecular analysis.

2.5. Nutrient analysis

Soil nitrate and ammonium were extracted with 0.01 M $CaCl_2$ and measured with a Bran Luebbe Flow Injection Autoanalyzer 3 System (SEAL Analytical Inc.). Soil Olsen phosphorus was extracted with 0.5 M sodium bicarbonate and measured with an ascorbic acid method (Watanabe and Olsen, 1965). Soil available potassium was extracted with 1 M ammonium acetate and determined on a flame photometer (FP6400A, Aopu).

2.6. Insecticide analysis

Insecticides were extracted and measured as described previously (Sun and Zeng, 2011; Sun et al., 2013). Briefly, 20 milliliter of 1% acetic acid in acetonitrile was added to a 50 mL Teflon centrifuge tube containing 10 g of homogenized vegetable samples (or soil samples). The tubes were shaken for 1 min using a vortex mixer. Then, six grams of anhydrous magnesium sulphate and 1.5 g of sodium acetate were added, and the tubes were shaken vigorously for an additional one min. The extract was centrifuged for five min at 5000 rpm at 4 °C, and two mL supernatant was transferred to another tube containing 50 mg PSA, 7.5 mg GCB, 50 mg C18 and 150 mg magnesium sulphate for vegetable samples and 50 mg Florifil, 50 mg C18 and 50 mg PSA for soil samples. After