Soil Biology & Biochemistry 114 (2017) 181–188

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Fertilization decreases compositional variation of paddy bacterial community across geographical gradient

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ARTICLE INFO

Article history:
Received 23 March 2017
Received in revised form
9 July 2017
Accepted 16 July 2017
Available online 24 July 2017

Keywords:
Fertilization
Soil microorganisms
β-diversity
Paddy soil
Soil fertility
Bacterial phylotypes

ABSTRACT

Fertilization is one of the most common agricultural practices to meet an increasing global demand for food products. Few investigations have been reported on spatial variation of microbial community composition in response to fertilizations in agroecosystems at a large scale. To improve the related understandings, we have evaluated the taxonomic and phylogenetic diversities of bacterial taxa in response to three fertilization strategies in six paddy experiment sites spanning across subtropical China. We found the large-scale compositional variation of paddy bacterial community is shaped by both geographic location and environmental selection, and the former is the dominant factor. The slopes of distance-decay relationships (DDR) are flattened by fertilizations, NPK (mineral NPK fertilizers) and OM (mineral NPK fertilizers plus organic amendments) when compared to Control. A flattened DDR implies that bacterial community composition is greatly homogenized by fertilizations in paddies. It is also inferred that fertilization decreases sensitivity of bacterial community to geographic and environmental factors, which is speculated to be beneficial for agroecosystem stability and yield sustainability. Results from this investigation correlate microscopic agroecosystem with macroscopic agricultural practices.

1. Introduction

One of the central themes of microbial ecology is to document, explain, and ultimately, predict the distribution of microorganisms in space and/or time (Hubbell, 2001). Spatial patterns of soil microbial community have been well documented in a variety of natural ecosystems, e.g. forest (Fierer and Jackson, 2006), grassland (Barnard et al., 2013), wetland (Bodelier et al., 2013), tundra (Chu et al., 2010) and etc. Meantime, various theories and conceptual models resulted from these natural ecosystems have been proven useful for understanding and predicting microbial biogeographical patterns (Martiny et al., 2011). The classic microbiological tenet "Everything is everywhere, but the environment selects" (Baas Becking, 1934) advocates that environmental selection is the primary driving force to shape soil microbial communities. The species pool hypothesis (Zobel, 1997) suggests that the species variation is mostly limited by regional species pool, which is mainly shaped by geographic locations, e.g., geological age, speciation, microbial dispersion, immigration and extinction. Before the neutral theory was developed (Kimura, 1968), environment selection had always been deemed more important than speciation, drift or dispersal of microorganisms which are mainly influenced geographic locations (Vellend, 2010). And this idea was popular when studying agroecosystems with anthropogenic control of soil environment. However, between the two major representative governing factors (geographic factor and local environmental selection) shaping taxonomic and phylogenetic variations of soil microorganisms (Ramette and Tiedje, 2007), their relative importances remain unclear. It wasn't until recent years have limited investigations been reported on anthropogenic-influenced agroecosystems, especially on the regional scale. Naturally, we are curious about the degree of contribution of environmental selection in influencing microbial communities in agroecosystems. Hence our curiosity drives this exploration.

The ultimate purpose of agroecosystem studies is to improve both quantity and quality of agricultural products, in order to meet an ever-increasing demand (Tilman et al., 2011). Nowadays, a heavy and continuous fertilization is a must for meeting such a demand.

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http://dx.doi.org/10.1016/j.soilbio.2017.07.013
0038-0717/

No one doubts that intensive fertilization poses influence onto soil microorganisms (Reganold et al., 2001). Concomitantly, it is not unexpected to find fertilization results in strong perturbation to the community compositions of bacteria (Feng et al., 2015) and fungi (Liu et al., 2015) at a single site (or local scale). In this investigation, we expanded our experiments to multiple sites across subtropical China, so we can systematically study how, at a regional scale, fertilization influences compositional variations of paddy bacterial community. Analysis of distance-decay relationship (DDR), including both geographic distance decay and environmental distance decay, is a powerful approach to deconvolute spatial compositional variation of microbial biodiversity and community assembly at different scales (Chu et al., 2016; Ramette and Tiedje, 2007). For example, DDR analysis found that the rate of change in microbial community composition across spatial distance is decreased by long-term oil contamination (Liang et al., 2015b). Elevated CO_2 accelerated the DDRs of soil microbial communities across disparate sites (Deng et al., 2016). Therefore, it is prudent to hypothesize that fertilization will alter the spatial compositional variation of microbial communities at a regional scale. Unfortunately, the exact extent of impacts remains largely unclear. We believe the advantages of DDR approach can be further extended in studying the response of soil microbial diversity to fertilization in croplands at a regional scale. By doing so, we hope to fill this knowledge gap, and eventually to be able to provide practical guidance for management decisions as well as predictions of ecosystem sustainability.

Paddy soils are anthropogenic soils widely distributed in subtropical Asia (Yan et al., 2003). China has nearly 34 million ha of paddy field across the subtropical zone, which yields ca. 40% of national food production. We had the opportunity of collecting paddy soil samples from six agro-ecological experimental sites across subtropical China to evaluate their spatial compositional variations of paddy soil microorganisms. These six experimental sites cover a wide geographic area (~1000 km), allowing an understanding of soil microbial diversity in an agro-ecosystem at a regional scale. On each site, there had been field fertilization experiments with identical fertilization strategies of Control (without fertilizers), NPK (with mineral N, P and K fertilizers) and OM (with organic amendments plus mineral NPK fertilizers). These sites have been under fertilization treatments for years. Therefore they produce ideal soil samples to study the variation of microbial community distributions driven by persistent anthropogenic activities. With Illumina high-throughput sequencing, we compared the soil microbial community compositions and their compositional variations (β-diversity) across six experimental sites with three fertilization strategies. Our investigation was based on two major hypotheses. Firstly, in anthropogenic paddy soils, we expect environmental factors to be dominant in shaping soil microbial communities. Secondly, we hypothesize that fertilization decreases spatial compositional variation of paddy bacterial community, driven by the continuous input of exogenous carbon and nutrients.

2. Methods and materials

2.1. Experimental sites and soil sampling

Soil samples were collected from six agro-ecological experimental sites across subtropical China (Fig. 1). Details of experimental sites were fully described in Table S1. Crow-fly distance of experimental sites is till about 1000 km. Mean annual air temperatures and mean annual precipitation was obtained from website of Weather China (http://www.weather.com.cn).

Each experimental site contained a fertilization plot experiment. Among the set fertilization strategies, we selected three treatments in the current study, Control (without fertilization), NPK (mineral NPK fertilizers) and OM (mineral NPK fertilizers plus organic amendments). Details about the design of the three treatments were listed in Table S2. There were six experimental sites included in this study. For each site, three replicate samples were extracted from each of the three fertilization treatments. Therefore, a total of 54 samples were analyzed.

Soil samples were collected after the harvest of paddy rice (for double cropping rice, after the late cropping) in 2014. Each soil sample was combined with 10 cores taken at a depth of 0–10 cm using a 30-mm-diameter gouge auger. Ten cores were mixed, coarse roots and stones were removed and then taken to the laboratory on ice. For each soil sample, one subsample was air-dried, sieved and used for analysis of chemical properties; the other subsample was used for DNA extraction.

2.2. Analysis of chemical properties

Soil pH was determined from soil-water suspensions (1:2.5 v/v). Soil organic C was determined by dichromate oxidization. Soil total N was determined by Kjeldahl digestion. Soil total P and K were first digested by hydrofluoric acid (HF)-perchloric acid ($HClO_4$) and then determined by molybdenum-blue colorimetry and flame photometry, respectively. Available P in the soil was extracted by sodium bicarbonate and determined using the molybdenum-blue method. Available K in the soil was extracted by ammonium acetate and determined by flame photometry.

2.3. DNA extraction

Genomic DNA was extracted from 0.5 g soil by using a FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA). The extracted DNA was dissolved in 50 μL TE buffer, quantified by spectrophotometer and quality evaluated by gel electrophoresis. After that, the extracted DNAs were evaluated by NanoDrop 2000 (ThermoFisher, USA) and stored at −20 °C until further usage.

2.4. PCR and high-throughput sequencing of 16S rRNA genes

PCR amplification was conducted for bacteria with primer set 519F/907R. The 5-bp bar-coded oligonucleotides of were fused to the forward primer. PCR was carried out in 50-μL reaction mixture, containing deoxynucleoside triphosphate at a concentration of 1.25 μM, 2 μL (15 μM) forward and reverse primers, 2 U of Taq DNA polymerase (TaKaRa, Japan), and each reaction mixture received 1 μL (50 ng) of genomic community DNA as a template. PCR reactions were performed according to the following program: 94 °C for 5 min, 30 cycles (94 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s), and a final extension at 72 °C for 10 min. Reaction products for each soil sample were pooled and purified using the QIAquick PCR Purification kit (Qiagen), and quantified using NanoDrop ND-2000 (Thermo Scientific, USA).

High-throughput sequencing was performed with Illumina Miseq sequencing platform (Illumina Inc.). The bar-coded PCR products from all samples were normalized in equimolar amounts before sequencing. After sequencing was completed, 16S rRNA genes data were processed using the Quantitative Insights Into Microbial Ecology (QIIME, USA) pipeline for data sets ((Caporaso et al., 2010), http://qiime.source.org). Sequences with a quality score below 25 and the length fewer than 200 bp were trimmed and then assigned to soil samples based on unique barcodes. Sequences were binned into OTUs using a 97% identity threshold, and the most abundant sequence from each OTU was selected as a representative sequence. Taxonomy was then