Science of the Total Environment 627 (2018) 20–27

Contents lists available at ScienceDirect

Science of the Total Environment

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

Biogeography and ecological processes affecting root-associated bacterial communities in soybean fields across China

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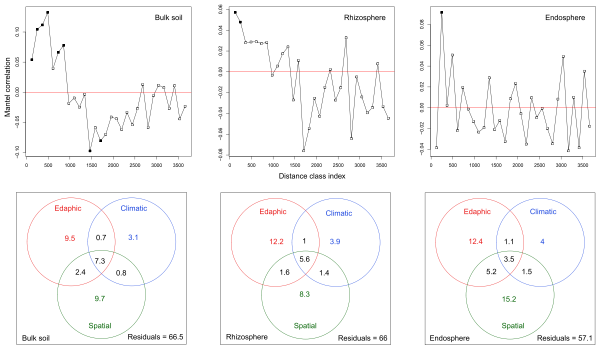
HIGHLIGHTS

- Bacterial communities in bulk soil, soybean rhizosphere and endosphere were compared.
- High–throughput sequencing was performed on an Illumina HiSeq 2500 platform.
- Environmental rather than spatial factors governed bacterial community turnover.
- Edaphic factors were more important than climatic factors for community turnover.
- Bacteria in the three compartments displayed different biogeographic patterns.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 November 2017
Received in revised form 23 January 2018
Accepted 23 January 2018
Available online xxxx

Editor: Elena PAOLETTI

Keywords:
Biogeography
Ecological processes
Bacterial community
Rhizosphere
Endosphere

ABSTRACT

Root-associated bacteria have profound effects on plant health and productivity, but their biogeographic patterns across large spatial scales remain poorly understood. Here, we used high–throughput sequencing to compare the bacterial distributions in the bulk soil, rhizosphere, and endosphere across 51 soybean fields in China. Environmental variables were more important than spatial variables, and edaphic variables were more important than climatic variables, for governing bacterial community turnover in each soil-root compartment. Both bacterial richness and community turnover were significantly correlated with different environmental and spatial variables among the three compartments. Their different spatial autocorrelation ranges for bacteria suggested distinct bacterial biogeographic patterns were present. The distributions of nearest taxon index (NTI) showed that deterministic processes dominated local bacterial communities, while its importance decreased from the bulk soil to the endosphere. These results provide new insights into the assembly of root–associated bacterial communities at a continental scale.

1. Introduction

Plant roots provide habitats for the diverse microorganisms in the rhizosphere and endosphere, where complex plant-microbe interactions play an essential role in plant nutrient uptake, disease suppression, and resistance to abiotic stress (Bulgarelli et al., 2013; Mendes et al., 2011; Reinhold-Hurek et al., 2015). Using next-generation sequencing technologies, numerous studies have investigated the composition and structure of root–associated bacterial communities and the main factors affecting them, such as soil types, plant species, and host developmental stage (Bulgarelli et al., 2012; Edwards et al., 2015;

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https://doi.org/10.1016/j.scitotenv.2018.01.230
0048-9697/

Lundberg et al., 2012; Xiao et al., 2017). However, only a few studies have explored the biogeographic patterns of root–associated bacterial communities (Fan et al., 2017; Nuccio et al., 2016).

Biogeography is the study of organism distribution patterns over space and time (Hanson et al., 2012). Gaining knowledge about the biogeographic patterns of microorganisms is particularly important because it can provide key insights into the mechanisms that generate and maintain microbial diversity (Martiny et al., 2006), which would help to better predict ecosystem-level responses to environmental change. Microbial biogeographic patterns are driven by local environmental factors (i.e., deterministic processes) and regional processes, such as dispersal limitations, mass effects, and historical factors (Hanson et al., 2012; Vellend, 2010; Wang et al., 2013). The bacterial biogeographic patterns in bulk soil have been extensively investigated in multiple ecosystems (i.e., both natural and agricultural) and at various spatial scales (i.e., local, regional, and global). Studies have revealed the importance of spatial factors (Caruso et al., 2011; Dumbrell et al., 2010) and environmental factors, such as soil pH (Fierer and Jackson, 2006; Griffiths et al., 2011), carbon content (Chu et al., 2016), C/N ratio (Högberg et al., 2007), precipitation (Angel et al., 2010), and temperature (Zhou et al., 2016), in shaping bacterial spatial distribution patterns. Nonetheless, current knowledge of bacterial biogeographic patterns in the rhizosphere and endosphere is relatively limited, such that bacterial biogeographic patterns have not been compared among the bulk soil, rhizosphere, and endosphere compartments.

More importantly, microbial biogeographic patterns and their causal ecological processes are scale-dependent (Levin, 1992; Talbot et al., 2014). For example, Martiny et al. (2011) demonstrated that geographic distance determines ammonia-oxidizing bacterial community composition at the local but not regional scale. However, most studies investigating root–associated bacterial communities have collected their samples from a small number of sites or from greenhouse systems. A notable exception is the study by Nuccio et al. (2016) spanning a > 500-km north–south gradient in California and that by Fan et al. (2017) across c. 1000 km of northern China. Nuccio et al. (2016) found that the wild oat rhizosphere communities were most influenced by climatic factors, while the bulk soil communities responded more to edaphic factors. Fan et al. (2017) showed that the bacterial communities in wheat rhizosphere were controlled more by geographic distance than by environmental factors. As both studies were performed at the regional scale, the root–associated bacterial distribution patterns at larger (i.e., continental) spatial scales remain largely undetermined.

Soybean (*Glycine* max) is a major economic crop and is widely distributed across China (Li et al., 2008). This enables sampling across a broad range of geographic locations and environmental gradients. Here, we used high–throughput sequencing to compare the bacterial communities of soybean in three distinct soil-root compartments (bulk soil, rhizosphere, and endosphere) in 51 fields across China. The objectives of this study were to determine and compare bacterial biogeographic patterns among the three soil–root compartments at a continental scale. We hypothesized that (i) bacteria in the three compartments show different biogeographic patterns; and (ii) the ecological factors and their relative importance in governing bacterial community turnover are different among the three compartments.

2. Materials and methods

2.1. Sample collection

Samples were collected from 51 soybean fields in China, these fields were located between 19°–50° N and 81°–130° E (Fig. 1). The distances between locations ranged from 63 to 3700 km. All fields were cultivated under conventional (i.e., pesticides and chemical fertilizers use permitted) but not organic (i.e., organic, manure, or compost fertilizers used) practices, and were planted with modern domesticated and locally adapted soybean cultivars. Sampling sites and relevant climate information are detailed in Supplementary Table S1. The sampling was conducted between May and August of 2015, which corresponds to the flowering period of the plant in all fields. In each field, a ~100 m^2 plot was chosen, and the bulk soil samples were taken at random from the topsoil layer (0–20 cm) in five cores (5 cm diameter) and pooled. Along with the surrounding soil, 15–20 randomly picked healthy plants were carefully removed from each field using a spade. All plants were taken from the central region of each field to avoid edge effects. Roots were carefully shaken to remove loosely attached soil, grouped into one sample per field and placed in plastic bags. All samples were transported to the laboratory on ice and stored at −80 °C until DNA extraction.

2.2. Acquisition of environmental data

A subset of the soil was air-dried and analyzed for its texture, organic C, pH, total/available N, and macronutrient contents (i.e., available P, K, Mg, and Ca) by using the standard soil testing procedures described in Bao (2000). Monthly weather data were extracted from the China Meteorological Database (http://data.cma.cn/). Climate data were collected from 51 weather stations near the sampling sites. Mean annual temperature (MAT) and mean annual precipitation (MAP) were computed based on the average monthly values. We also calculated one, two, and three month sum precipitation (SMP1–3) and mean temperature (MMT1–3) ranges.

2.3. Sample preparation, DNA extraction and sequencing

The rhizosphere and endosphere compartments were separately sampled by sequential washing and sonication treatments following a previously described method (Lundberg et al., 2012; Xiao et al., 2017). A detailed description of how the different compartments were separated in this study is provided in the Supplementary Information. Nodules were removed from the endosphere compartment and a 0.5 g portion of soil (bulk or rhizosphere) was used for DNA extraction. For endosphere samples, ~1 g of roots were lysed by grinding under liquid nitrogen using a mortar and pestle. The DNA for each sample was extracted using the Fast DNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's protocol. DNA concentration and purity was confirmed on 1% (w/v) agarose gels. The V4 region of the bacterial 16S rRNA gene was amplified using the 515F (5′-GTGCCAGC MGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers (Evans et al., 2014) with unique barcodes. All PCR reactions (30 μL volume) contained: 15 μL Phusion Master Mix (New England Biolabs), 0.2 μM forward and reverse primers, and 10 ng template DNA. The DNA from each sample was individually amplified by PCR in triplicated reactions consisting of: initial denaturation at 98 °C for 1 min, 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s, and a final elongation at 72 °C for 5 min. Triplicate PCR products were pooled and purified with the Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing libraries were generated by using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's recommendations with index codes. Sequencing (250 bp paired end) was run on an Illumina HiSeq 2500 at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

2.4. Sequence analysis

Paired-end reads were merged using FLASH (Magoc and Salzberg, 2011) and assigned to each sample according to the unique barcodes using QIIME (Caporaso et al., 2010). Sequences <200 bp in length, with average quality <25, or containing ambiguous bases were discarded. Chimeras were detected and removed using UCHIME (Edgar et al., 2011). High-quality sequences with ≥97% similarities were then assigned to the same operational taxonomic unit (OTU)