Soil Biology & Biochemistry 90 (2015) 10–18

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Environmental conditions rather than microbial inoculum composition determine the bacterial composition, microbial biomass and enzymatic activity of reconstructed soil microbial communities

Weibing Xun [a, b], Ting Huang [c], Jun Zhao [a], Wei Ran [a], Boren Wang [d], Qirong Shen [a], Ruifu Zhang [a, b, *]

[a] National Engineering Research Center for Organic-based Fertilizers, Jiangsu Key Lab and Engineering Center for Solid Organic Waste Utilization, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Key Laboratory of Microbial Resources Collection and Preservation, Ministry of Agriculture, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China
[c] College of Sciences, Nanjing Agricultural University, Nanjing 210095, PR China
[d] Qiyang Red Soil Experimental Station, Chinese Academy of Agricultural Sciences, Qiyang 426182, PR China

ARTICLE INFO

Article history:
Received 26 December 2014
Received in revised form
17 July 2015
Accepted 22 July 2015
Available online 4 August 2015

Keywords:
γ-ray sterilization
Soil incubation
Bacterial community
Microbial biomass
Enzymatic activity

ABSTRACT

The composition of microbial communities and the level of enzymatic activity in the soil are both important indicators of soil quality, but the mechanisms by which a soil bacterial community is generated and maintained are not yet fully understood. Two soil samples were collected from the same location, but each had been subjected to a different long-term fertilization treatment and was characterized by different microbial diversity, biomass and physicochemical properties. These samples were γ-sterilized and swap inoculated. Non-sterilized soil samples along with sterilized and inoculated soil samples were incubated for eight months before their nutrient content, microbial biomass, enzymatic activity and bacterial composition were analyzed. Total phosphorus, and potassium concentrations along with the overall organic matter content of the non-sterilized soil were all equal to those of the same soil that had been sterilized and self/swap inoculated. Additionally, the microbial biomass carbon concentration was not affected by the specific inoculum and varied only by soil type. The activities of catalase, invertase, urease, protease, acid phosphatase and phytase were smaller in the sterilized soils that had been inoculated with organisms from chemical fertilizer amended soil (NPK) when compared to sterilized soil inoculated with organisms from manure and chemical fertilizer amended soil (NPKM) and non-sterilized soil samples. Bacterial 16S rRNA examined by 454-pyrosequencing revealed that the composition of bacterial community reconstructed by immigrant microbial inoculum in the soil was mainly influenced by its physicochemical properties, although the microbial inoculum contained different abundances of bacterial taxa. For example, the pH of the soil was the dominant factor in reconstructing a new bacterial community. Taken together, these results demonstrated that both soil microbial composition and functionality were primarily determined by soil properties rather than the microbial inoculum, which contributed to our understanding of how soil microbial communities are generated and maintained.

1. Introduction

Biological activity in the soil is primarily dependent on ecosystem processes that are supported by microbes (Daily, 1997), which are especially important in maintaining the magnitude and stability of nutrient cycling (Weyhenmeyer et al., 2013). Therefore, identifying the mechanism by which microbial communities are generated and maintained in the soil is essential for understanding its biological functions.

In general, organisms that arrive in a new environment either by being artificially transported or by natural dispersal need to cope with two main stresses: environmental changes and novel interactions with local biological communities (Gómez et al., 2010).

* Corresponding author. College of Resources & Environmental Science, Nanjing Agricultural University, Nanjing, 210095, China. Tel.: +86 25 84396477; fax: +86 25 84396260.
E-mail address: rfzhang@njau.edu.cn (R. Zhang).

http://dx.doi.org/10.1016/j.soilbio.2015.07.018
0038-0717/

Bacteria utilize a range of strategies to adapt to their new environment, including the development of morphological plasticity (Justice et al., 2008), the utilization of an arginine deaminase system in response to an acid environments (Marquis et al., 1987) and the interaction with phagocytic cells in response to oxidative stress (Hassett and Cohen, 1989).

A century ago microbial biogeography was described in the following manner: "Everything is everywhere, the environment selects" (Beijerinck, 1913); however, recent thinking has challenged this idea. Organisms become dormant when they faced with unfavorable environmental conditions (Jones and Lennon, 2010). These unfavorable environmental conditions including deviation from optimum pH values, poor soil nutrient, high or low temperatures, toxic substances, etc. are survival challenges for organisms. Understanding these challenges is important for in situ microbial community reconstruction.

Dormant microorganisms generate a microbial inoculum (Lennon and Jones, 2011), which is responsible for the diversity and dynamics of communities in the future. Previous study (Cruz-Martínez et al., 2009) has addressed in situ changes in the composition of microbial communities that have the same original microbial community, but the introduction of an entirely new microbial inoculum into a novel environment, especially a sterilized one, has not yet been well studied.

Nannipieri et al. (1983) suggested that each soil has its own 'biological space' and therefore maintains a specific microbial biomass value under conditions of equilibrium. Soil type might be the determinant which tend to maintain the composition of bacterial communities (Girvan et al., 2003). Griffiths et al. (2008) established a swapping microbial community of sandy and clay-loam soil and reported that the composition of a microbial community depends on soil type rather than the source of the inoculum. This was confirmed by Delmont et al. (2014), who identified the composition of the bacterial communities found in two different sterilized soils that had been inoculated with either a non-sterilized sample from the same soil or with a non-sterilized foreign soil sample. However, these experiments used essentially the different soils with different parent materials and different vegetations. Moreover, the new microbial activity in these reconstructed microbial communities was not examined, and this information may provide insight into the relationship between microbial diversity and microbial functions.

We hypothesized that not only the soil bacterial composition but also the soil microbial functionality was primarily determined by soil properties rather than the microbial inoculum. To test this, two soil samples of the same origin but different in fertilization management over the previous 23 years were used. Their microbial communities were swap-inoculated and the composition of the reconstructed bacterial communities was determined by barcode pyrosequencing of the 16S rRNA gene (Borneman and Triplett, 1997). Functional gene abundances were detected using quantitative real-time PCR. Soil enzymatic activity, physicochemical properties and microbial biomass were also assessed.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected from the Red Soil Experiential Station (RSES) maintained by the Chinese Academy of Agricultural Sciences, Qiyang (111°53′E, 26°45′N), Hunan Province, southern China, which is located at an altitude of 120 m. The soil at this station is known as Ferralic Cambisol which originally developed from Quaternary red clay. Fertilization experiment began in 1990 and included annual rotations of winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.). Different fertilization treatments were implemented with two replicates in a random block design (Chen et al., 2014). Soil samples were collected in November 2011 from two fertilization treatments, i.e. chemical fertilization (nitrogen, phosphate, and potassium fertilizer, NPK) and manure chemical combined fertilization (NPKM). Fresh samples of each treatment were obtained from the two replicated plots (8 random soil cores per plot) and mixed thoroughly for further study. Both soil samples were passed through a 2 mm sieve after having been temporarily preserved in a portable storage box and transported to the lab. Subsamples that were used for measuring enzymatic activity and physicochemical properties were air-dried, subsamples used for DNA extraction were stored at −80 °C, and others subsamples were temporarily stored at 4 °C for further study.

2.2. Microbial community swapping and incubating

After soil samples stored at 4 °C were sterilized by gamma ray (60 kGy) irradiation, a 200 g portion sterilized soil was placed into a 500 mL bottle and allowed to stabilize for 8 weeks at room temperature (Ramsay and Bawden, 1983). Next, sterile water was added to maintain a constant moisture level (30% of field capacity), and the samples were pre-incubated at 20 °C in the dark for 2 weeks before being tested for sterility. Microbial inocula were prepared from both NPK and NPKM amended soil samples that had been stored at 4 °C. First, the soil samples were pre-incubated at 20 °C in regularly aerated bags for two weeks. Next, 20 g of soil was placed in 180 mL of sterile water along with 20 g of glass beads (3–4 mm in diameter) and shaken for 20 min. After 20 mL of this soil suspension was mixed with 200 g of γ-ray sterilized soil, microbial inocula from NPK amended soil were introduced into sterilized soil samples from both the NPK (self inoculation control, designated as NPKtoNPK) and NPKM (designated as NPKtoNPKM) groups. Conversely, microbial inocula from NPKM amended soil were introduced into sterilized soil samples from both the NPKM (self inoculation control, designated as NPKMtoNPKM) and NPK (designated as NPKMtoNPK) groups. In addition, 200 g of non-sterilized NPK and NPKM soil samples that had been stored at 4 °C were placed into a sterilized 500 mL bottle to serve as controls (designated as CKNPK and CKNPKM, respectively). Three replicates of each control, self inoculation control and swap inoculation treatment were randomly blocked and incubated at 20 °C at constant moisture (45% of field capacity) for eight months (2012.01–2012.08).

2.3. Soil analysis

After eight-month incubation, the soils were passed through a 2 mm sieve, and several measurements were taken. Soil pH was assessed with a PHS–3C mv/pH detector (Shanghai, China) at a soil-to-water ratio of 1:5, available P (AP) was extracted with sodium bicarbonate and its concentration was determined by the molybdenum blue method (Olsen et al., 1954), available K (AK) was extracted with ammonium acetate and its concentration was determined by flame photometry (Dahnke, 1988), total N (TN) was determined by Kjeldahl digestion (Bremner and Mulvaney, 1982), total P (TP) and total K (TK) were isolated by HF–$HClO_4$ (Jackson, 1958) and their concentrations were determined by molybdenum-blue colorimetry and flame photometry respectively, soil organic matter (SOM) concentrations were determined by the potassium dichromate volumetric method (Schollenberger, 1931) and microbial biomass carbon (MBC) concentrations were determined by the chloroform fumigation-extraction method (Vance et al., 1987).