Soil Biology and Biochemistry 122 (2018) 111–119

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

Long-term effects of manure and chemical fertilizers on soil antibiotic resistome

Wan-Ying Xie[a], Shuang-Ting Yuan[a], Ming-Gang Xu[b], Xin-Ping Yang[a], Qi-Rong Shen[a], Wen-Wen Zhang[a], Jian-Qiang Su[c], Fang-Jie Zhao[a,*]

[a] Jiangsu Provincial Key Laboratory for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China
[b] Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[c] Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

ARTICLE INFO

Keywords:
Antibiotic resistance genes
Bacterial community
Long-term fertilization
NPK'
Manure
Horizontal gene transfer

ABSTRACT

Applications of manure and chemical fertilizers can significantly modulate the bacterial communities in soil, but the effects on the soil antibiotic resistome and the underlying mechanisms remain largely unclear. In the present study, antibiotic resistance genes (ARGs) and bacterial communities were characterized using high-throughput quantitative PCR and Illumina HiSeq sequencing, respectively, in soils from a long-term (25 years) field experiment. Treatments included no fertilizers (control), chemical fertilizers (NPK), NPK with straw return (NPKS), pig manure (M), and M with chemical fertilizers (NPKM). Both NPK and NPKS decreased soil pH and induced a substantial shift in the bacterial communities, but had only moderate effect on the ARG diversity and abundance. The effect of straw return was hindered by the dominant influence of NPK. In comparison, additions of pig manure (M and NPKM) maintained the diversity of bacterial community and significantly influenced the ARG profiles by introducing extra ARGs from pig manure and boosting the soil indigenous ARG members. Manured soils harboured clusters of ARGs and transposase genes which were relatively independent of bacterial phylogenetic lineages, suggesting a strong co-occurrence of ARGs in soil bacteria which may result from horizontal gene transfers (HGT). Our results indicate the importance of HGT in the maintenance of ARG composition in agricultural soils, especially those receiving long-term manure applications.

1. Introduction

Soil is a natural source of antibiotics and antibiotic resistance genes (ARGs) (Allen et al., 2010; Nesme and Simonet, 2015). ARGs in soils are important constituents of the environmental resistome, which plays a vital part in influencing the resistome of human pathogens (Forsberg et al., 2012; Gillings, 2013). Indigenous groups of ARGs can be found in soil bacterial communities without any anthropogenic influence (Allen et al., 2008; D'Costa et al., 2011; Martinez et al., 2015). Apart from the indigenous members, soil bacteria can acquire resistance from exogenous sources, such as the frequent observations of ARG introduction from animal manures into agricultural soils (Graham et al., 2016; Heuer et al., 2011a, 2011b; Peng et al., 2017; Zhu et al., 2013).

Recycling animal manures to agricultural soils is an important way of nutrient management that can improve soil fertility (Steiner et al., 2007). However, due to the widespread use of antibiotics in the treatment and prevention of diseases and as feed additives for growth promotion in animal industries, substantial amounts of unabsorbed antibiotics and the concomitant ARGs have been unintendedly introduced into the soil environment following manure applications (Johnson et al., 2016; Wang et al., 2013; Zhou et al., 2017; Zhu et al., 2013). The ARGs introduced in the manure can be recruited by soil bacteria as members of the soil antibiotic resistome through the process of horizontal gene transfer (HGT), enhancing both the abundance and diversity of ARGs (Klumper et al., 2015; Srinivasan et al., 2008; You et al., 2012). In addition, manure applications may increase the potential of lateral gene exchanges between bacteria through HGT (Graham et al., 2016). More worryingly, common ARG members shared by soil bacteria and human pathogens have been detected by functional metagenomics, indicating the clinical importance of antibiotic resistance in soils (Forsberg et al., 2012).

The various ARGs in the environments are hosted by diverse commensal or pathogenic bacteria (Martinez et al., 2015). Therefore, it is possible that alterations of the bacterial community could also influence the ARG profile. A number of previous studies have shown that bacterial phylogeny and taxonomic structure significantly correlate

* Corresponding author.
E-mail address: Fangjie.Zhao@njau.edu.cn (F.-J. Zhao).

https://doi.org/10.1016/j.soilbio.2018.04.009
Received 25 November 2017; Received in revised form 8 April 2018; Accepted 9 April 2018

with resistome composition in sludge composts and soils (Chen et al., 2016; Forsberg et al., 2014; Peng et al., 2017; Su et al., 2015). Applications of animal manures can affect soil bacterial diversity, composition and structure (Steiner et al., 2007; Sun et al., 2015). There is some evidence that the long-term influence of manure on soil resistome is primarily through the alteration of bacterial composition rather than direct introduction of manure-borne bacteria (Chu et al., 2007; Sun et al., 2015; Xun et al., 2016). Long-term applications of chemical fertilizers can cause dramatic alterations of soil properties and bacterial community (Sun et al., 2015; Xun et al., 2016). However, little information is available with regard to how variations in the bacterial community impact the soil ARG resistome in the context of long-term applications of animal manure and chemical fertilizers.

The objective of the present study was to understand how the soil resistome and bacterial community react to long-term applications of animal manure and chemical fertilizers and the relationship between them. We hypothesize that organic matter-rich animal manure that contains ARGs and possibly also antibiotics influences both the soil bacterial community and the resistome differently from chemical fertilizers. To test this hypothesis, we profiled the bacterial communities and ARGs in soils receiving different treatments of manure and chemical fertilizers for 25 years, and showed that although there were significant correlations between soil ARG composition and structures of bacterial taxonomy, ARG profiles in manured soils were predominantly influenced by the exogenous introduction of ARGs and the process of HGT.

2. Materials and methods

2.1. Experimental treatments and soil sampling

The field experiment was established in Qiyang Red Soil Experimental Station (111°53′E, 26°45′N), Hunan Province, China, in 1990 and has continued since. The soil is classified as Ferralic Cambisol derived from quaternary red clay (Xun et al., 2016; Zhang et al., 2009). The site is located in the subtropical region with a typical cropping system of maize-wheat rotation (He et al., 2007; Zhang et al., 2013). The experimental design and fertilization scheme have been described in details in previous studies (He et al., 2007; Xun et al., 2016; Zhang et al., 2009). We chose 5 of the 12 treatments, including: (1) control (no fertilizers since 1990); (2) chemical fertilizers (nitrogen, phosphorous and potassium; NPK); (3) chemical fertilizers and straw incorporation ((NPKS); (4) fresh farmyard pig manure (M); (5) chemical fertilizers and fresh farmyard pig manure (NPKM). The rates of fertilizers, manure and straw are shown in Table 1. Thirty percent of the fertilizers were applied in the wheat season and the other 70% in the maize season, both prior to sowing of the crops (Chen et al., 2014). Each treatment contained two plots that were 20 m × 10 m separated by 100-cm-deep cement plates. The two plots were divided into four sub-plots longitudinally for sampling, but only three of them were selected randomly as three replicates for further analyzes. Six cores (0–20 cm) were collected by auger from each sub-plot and bulked as one replicate. All samples were stored in sterile plastic bags, placed on ice and transported to the lab as soon as possible. Subsamples for the determination of chemical properties were air-dried, passed through a 0.15-mm sieve and stored at 4 °C. Subsamples for DNA extraction were freeze-dried, passed through a 2.0-mm sieve and stored at −80 °C.

2.2. Determination of soil properties

Soil pH was measured in a suspension of 1:5 soil: water using a pH meter (PB-10, Sartorius, Germany). Soil organic carbon (SOC) and total nitrogen (TN) were determined using a CN analyzer (Varia EL, Elementar GmbH, Hanau, Germany). The concentrations of P, K, Ca, Mg, Cu and Zn were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer NexIon 300x, Wellesley, MA) after soils were digested with aqua regia (HCl: HNO_3, V: V = 4: 1).

2.3. DNA extraction and purification

DNA was extracted from approximately 0.25 g of freeze-dried soil with a Powersoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). Each sample was extracted twice and the extracted DNA solutions were combined. The raw DNA samples were purified to remove PCR inhibitors. Briefly, the extracted DNA was incubated with 0.1 volume of 3 M sodium acetate (pH = 5.2) and 2.5 volume of ethanol at −20 °C overnight. The DNA pellets were obtained by centrifugation at 16,000 g at 4 °C for 1 h. The pellet was washed with 70% ethanol and re-dissolved in an elution buffer (10 mM Tris) of the Isolation Kit. Quality of the DNA was checked with the NanoDrop 2000C spectrophotometer. DNA concentrations were determined using a Quant-iT PicoGreen double-stranded DNA (dsDNA) assay kit (Invitrogen, USA). The DNA were stored at −20 °C until further analysis.

2.4. Characterization of bacterial community

The primer set 515 F (5′- barcode-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) was used to amplify the V4-V5 regions of the 16S rRNA gene (Xiong et al., 2012). The reaction was conducted on an ABI GeneAmp PCR System 9700 DNA thermal cycler (Applied Biosystems, Foster City, CA, USA) as described previously (Wang et al., 2016). The amplicons were excised from 2% agarose gels, purified with AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.), quantified using Qubit®3.0 (Life Invitrogen, Carlsbad, CA, USA), combined in equimolar and paired-end sequenced (2 × 250) on an Illumina HiSeq platform (Biozeron, Shanghai, China). QIIME 1.17 was used for read assembly after filtering the raw data by removing the adaptor, ambiguous nucleotides, low-quality reads and barcodes as described previously (Shi et al., 2017). The generated clean data were analyzed with QIIME 1.9 based on an open-reference OTU (operational taxonomy units) picking according to the online instruction with default settings (Caporaso et al., 2010; Su et al., 2015). The OTUs picking (97% similarity) and taxonomy assignment (confidence threshold of 80%) were performed using UCLUST and RDP classifier, respectively (Su et al., 2015). Chimeric

Table 1
Fertilization scheme in different treatments.

Treatment	Urea (kg ha $^{-1}$)	Calcium superphosphate (kg ha $^{-1}$)	Potassium chloride (kg ha $^{-1}$)	Fresh swine manure (Mg ha $^{-1}$)	Straw	pH	SOC (%)
Control	0	0	0	0	0	5.69 (0.26) b	0.86 (0.01) d
NPK	651	997	200	0	0	4.13 (0.13) c	1.06 (0.03) c
NPKS	651	997	200	0	Half straw return	4.09 (0.10) c	1.08 (0.01) c
M	0	0	0	60.0	0	6.64 (0.13) a	1.65 (0.04) a
NPKM	195	997	200	41.7	0	5.84 (0.09) b	1.45 (0.03) b

SOC, soil organic carbon.
Multiple comparisons were conducted between treatments using SPSS 18.0 at the significant level of 0.01.