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Responses of soil bacterial community after seventh yearly applications of composted tannery sludge

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ABSTRACT

Composted tannery sludge (CTS) contains organic compounds and inorganic elements, mainly chromium (Cr), and its long-term application in soil can alter the bacterial structure and diversity. Thus, we used the next-generation sequencing to assess the structure and diversity of bacterial communities in soils after 7 years of CTS application. CTS was applied at 0, 2.5, 5, 10, and 20 Mg ha^{-1} and the soil samples were collected at 75 days after application in the seventh year. The most abundant phyla were Actinobacteria, Proteobacteria, Firmicutes, and Chloroflexi. The abundance of some specific groups increased with application of CTS, such as Anaerolinea S0208 and Firmicutes. Six bacterial genera differed between amended and unamended soil. The abundance of *Bacillus*, *Paenibacillus*, *Symbiobacterium*, *Clostridium*, *Microlunatus*, and *Actinomadura* increased after application of CTS. The Redundancy Analysis between the structure of the bacterial community and chemical variables in soil did not cluster all treatments clearly, but showed Cr, pH, and organic C as significant chemical variables that influenced the bacterial communities. Application of CTS in soil has a primary effect on the bacterial communities that, negatively, alter the bacterial diversity and community similarity, while that, positively, it permits to select specific bacterial groups able to resist and biodegrade contaminants.

1. Introduction

The high generation of solid wastes has led to environmental concerns worldwide since they are disposed in landfilling and, therefore, posing risk to the environment (Srivastva et al., 2016). Agricultural use of solid waste has increased recently, being one of the most promising alternatives for its disposal (Araujo et al., 2010). However, these wastes can contain pathogens and organic and inorganic elements, such as heavy metals, which could lead to soil pollution in the long-term use (Hargreaves et al., 2008). Specifically, tannery sludge (TS), a solid waste produced by tannery industries, contains organic compounds and inorganic elements, such as chromium (Cr), salts, carbonates, and hydroxides (Araujo et al., 2015) that can accumulate in soil and cause environmental pollution.

The composting can be an alternative biological process for recycling and decomposition of TS before its agricultural utilization (Santos et al., 2011). It is also important since organic compost has potential of improving soil properties (Yuksel, 2015). In fact, previous studies have shown that the use of composted tannery sludge (CTS) has improved the physical and chemical properties of soil (Araujo et al., 2013; Araujo et al., 2016).

On the other hand, a concern about using CTS in agricultural soils is related with the soil biological properties, mainly soil microorganisms. Soil microorganisms are the key organisms involved in important ecological processes, such as nutrient cycling, and are known as the largest reservoirs of biodiversity (De Mandal et al., 2015). Also, soil microorganisms present biotechnological potential (Sleator et al., 2008) and are recognized as sensitive indicators of soil health and anthropogenic disturbances (Nakatani et al., 2011). Several previous studies were already carried out to assess the effect of CTS application on soil microorganisms in both short- and long-term (Santos et al., 2011; Miranda et al., 2014; Araujo et al., 2015; Araujo et al., 2016). In these studies,

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the responses of soil microorganisms and microbial process were contradictory, i.e. soil microbial biomass and activity showed negative responses; nodulation and nitrogen fixation responded positively, while enzymatic activity showed no response.

It is unclear how the long-term use of CTS affects the structure and diversity of microbial community and what the chemical factors are driving these effects. Although some studies have evaluated the effect of untreated TS on microbial communities in the short-term (Nakatani et al., 2011; Giordano et al., 2016), the responses of microbial structure and diversity to a long-term (years of) exposure due to successive applications of CTS has not been assessed.

Nowadays, the sequencing of 16S rRNA genes recovered from the environment is the most frequently used molecular method for evaluating bacterial diversity (Mishra et al., 2014) and the next-generation high-throughput sequencing method presents high accuracy to elucidate the structure of bacterial communities. In the last years, this technique has been used to study soil bacterial structure and diversity from several environments (Neelakanta and Sultana, 2013; De Mandal et al., 2015; Araujo et al., 2017) but it has not yet been used to evaluate the effects of application of composted wastes to soil bacterial communities.

In this study, we hypothesize that (1) the long-term applications of CTS alter the bacterial structure and diversity; and (2) specific chemical parameters drive the response of bacterial communities to CTS application. To test these hypotheses, we used the next-generation high-throughput sequencing method on Illumina Miseq in order to assess the structure and diversity of bacterial communities in soils after 7 years of CTS application.

2. Material and methods

2.1. Sampling site and experimental procedure

The experimental site is located at the Long-Term Experimental Field belonging to the Agricultural Science Center, Federal University of Piauí, Brazil. The soil is classified as Fluvisol with the following composition at 0–20 cm depth: 10% clay, 28% silt, and 62% sand. CTS was produced by mixing TS with sugarcane straw and cattle manure (ratio 1:3:1; v:v:v) and the composting was performed using the aerated-pile method (USDA 1980) for 90 days. The physicochemical characteristics of CTS were evaluated at the end of composting process (Table 1). The water content was determined after oven drying the samples at 105 °C for 24 h. The pH was directly measured by mixing CTS with water (1:2.5 v:v) using a pH-meter, and total solids were measured by drying the samples at 65 °C (APHA, 2005). The total organic C content was evaluated by dichromate oxidation of the samples under external heating (Nelson and Sommers, 1996). The total N content was determined using the Kjeldahl method after sulphuric acid digestion of the samples (Bremner, 1996). The total Ca, Mg, K, P, S, Na, Zn, Cu, Cd, Pb, Ni, and Cr concentrations were determined by atomic absorption spectrophotometry after nitric acid digestion of the samples in a microwave oven (USEPA, 1996).

CTS has been applied annually since 2009 at five rates: 0 (without CTS application), 2.5, 5, 10, and 20 Mg ha^{-1} of CTS (dry basis). The experimental site is arranged in a completely randomized design with four replicates for each treatment. Plots are 20 m^2 each, with 12 m^2 of usable area for soil and plant sampling, and rows are spaced 1.0 m apart. In the seventh year (2015), CTS was applied 10 days before maize (*Zea mays* L.) sowing. At application, the CTS was spread on the soil surface and incorporated into the 20 cm layer with a harrow. Maize was grown at a density of 5 plants m^{-1} (approximately 62,000 plants ha^{-1}) for 75 days. CTS was spread on the soil surface and incorporated into the 20 cm layer with a harrow. Soil samples were collected, in 2015, from each plot at 75 days after CTS application. For soil sampling, four samples were collected in each plot (0–20 cm), sieved (2-mm), and stored at 4 °C prior to analysis.

Table 1
Composition of composted tannery sludge.

	Moisture %	pH	TOC	N	P	K	Ca	Mg	Na	Cr
			g kg^{-1}							mg kg^{-1}
	68	7.5	201	15	4.9	2.9	121	7.2	49.1	1943
MLP[1]	–	–	–	–	–	–	–	–	–	150

MLP - maximum limit permitted (CONAMA, 2009); TOC - total organic carbon.

The soil samples for chemical analysis were air dried and sieved (2-mm). Soil pH, exchangeable Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}, and the available P were estimated according to EMBRAPA (1997). Soil electric conductivity (EC) was evaluated in water (1:2 v:v) according to the method described by Richards (1954). Total organic C (TOC) was determined by wet combustion using a mixture of 5 mL of 0.167 mol L^{-1} potassium dichromate and 7.5 mL of concentrated sulfuric acid under heating (170 °C for 30 min) (Yeomans and Bremner, 1988).

2.2. DNA extraction and sequencing

Soil DNA was extracted from 0.5 g (total humid weight) of soil using the Power Lyzer Power Soil DNA Isolation Kit (MoBIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The DNA extraction was performed in triplicate for each soil sample. The quality and concentration of the extracted DNA was determined using NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, USA).

The V4 region of the 16S rRNA gene was amplified with region-specific primers (515F/806R) (Caporaso et al., 2011). Each 25 μL PCR reaction contained the following: 12.25 μL of nuclease-free water (Certified Nuclease-free, Promega, Madison, WI, USA), 5.0 μL of buffer solution 5 × ($MgCl_2$ 2 Mm), 0,75 μL de solution of dNTP's (10 mM), 0,75 μL of each *primer* (515 YF 40 μM e 806 R 10 μM), 1.0 unit of Platinum Taq polymerase High Fidelity in concentration of 0,5 μL (Invitrogen, Carlsbad, CA, USA), and 2.0 μL of template DNA. Moreover, a control reaction was performed by adding water in place of DNA. The conditions for PCR were as follows: 95 °C for 3 min to denature the DNA, with 35 cycles at 98 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s, with a final extension of 3 min at 72 °C to ensure complete elongation.

After indexing, the PCR products were cleaned up using Agencourt AMPure XP - PCR purification beads (Beckman Coulter, Brea, CA, USA), according to the manufacturer's manual, and quantified using the dsDNA BR assay Kit (Invitrogen, Carlsbad, CA, USA) on a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). Once quantified, equimolar concentrations of each library were pooled into a single tube. After quantification, the molarity of the pool was determined and diluted to 2 nM, denatured, and then diluted to a final concentration of 8.0 pM with a 20% PhiX (Illumina, San Diego, CA, USA) spike for loading into the Illumina MiSeq sequencing machine (Illumina, San Diego, CA, USA).

2.3. Data processing

Sequence data were processed using QIIME following the UPARSE standard pipeline according to Brazilian Microbiome Project ((http://www.brmicrobiome.org/#!16s-profiling-pipeline-illumina/czxl) (Pylro et al., 2014), to produce an OTU table and a set of representative sequences. Briefly, the reads were truncated at 240 bp and quality-filtered using a maximum expected error value of 0.5. Pre-filtered reads were dereplicated and singletons were removed and filtered for additional chimeras using the RDP_gold database using USEARCH 7.0. These sequences were clustered into OTUs at a 97% similarity cutoff following the UPARSE pipeline. After clustering, the sequences were aligned and taxonomically classified against the Greengenes database (version