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Zinc concentration affects the functional groups of microbial communities in sugarcane-cultivated soil

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ABSTRACT

The addition of zinc (Zn) to the soil has significant gains in sugarcane productivity. The understanding of the consequences of zinc application requires insight into ecological aspects of changes in soil microbial communities. Here, we evaluated the effects of zinc application on richness, diversity, evenness and structure of microbial functional genes in sugarcane-cultivated soil, and response thresholds of abundance for groups of microbial functional genes on different zinc concentrations added to the soil (0, 5, 10 and 20 kg Zn ha^{-1}) in two sugarcane fields in the Northeastern São Paulo state, Brazil. Using a high-density functional gene array termed GeoChip 5.0, which contains 101,796 distinct probes belonging to gene categories involved in zinc transport, secondary metabolism, stress, virulence and nutrient cycling, differences were observed in the functional gene structure of microbial communities and abundance of specific gene families with a threshold between 5 and 10 kg Zn ha^{-1}. However, the richness, diversity and evenness of functional genes did not differ across the zinc gradients in soil. Cluster analysis based on microbial functional subcategories revealed variation in abundances across the gradient of zinc concentrations in soil mainly for virulence, stress, secondary metabolism, and carbon- and phosphorus-cycling-related gene families. A threshold in abundance was observed between 5 and 10 kg Zn ha^{-1} with high abundance of microbial gene families associated with zinc transporter proteins, antioxidant enzymes, exoenzymes associated with infection, secretion proteins, virulence regulatory genes, carbon fixation, and phosphorus utilization in soils supplemented with up to 5 kg Zn ha^{-1}. In these same soils, reduced abundance was observed for gene families associated with methanogenesis, metabolism of halogen and carotenoid, and antiphagocytosis compared with soil supplements with 10 or 20 kg Zn ha^{-1}. The results suggest that changes in microbial functional gene structure and abundance could be used to evaluate the impact of soil management practices on soil microbial communities in sugarcane production fields.

1. Introduction

Zinc is an essential cofactor required by all living organisms. Zinc is the second most abundant transition metal in living organisms (after iron), has important functions in enzymatic activity, transcription factors (which regulate gene expression), and serves as a cofactor in more than 300 proteins (Palmgren et al., 2008). According to a global study of the micronutrient status of soils (Sillanpää, 1990), the zinc content in soils from Brazilian regions are highly variable, covering a wide range of values from high to low, potentially leading to zinc deficiency for important crops cultivated in this area (e.g., sugarcane, coffee, oranges, rice, maize, sorghum, cassava and rubber). In this sense, several studies have demonstrated significant gains in sugarcane productivity

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with zinc application (Cambria et al., 1989; Marinho and Albuquerque, 1981; Korndörfer et al., 1995; Wang et al., 2005; Mellis et al., 2016).

The number of zinc-binding proteins correlates linearly with the total number of proteins encoded by the genome of an organism, but the proportionality constant of Eukaryota (8.8%) is significantly increased compared with that observed in Bacteria and Archaea (from 5% to 6%) due to the larger portfolio of regulatory proteins in Eukaryota (Andreini et al., 2006). Zinc levels must be tightly regulated; too little zinc does not support cellular growth, whereas too much zinc is cytotoxic (Koh et al., 1996; Costello et al., 1997). The free zinc concentration in the cytoplasm is maintained well below the picomolar range (Outten and O'Halloran, 2001). It is generally accepted that accumulated trace metals reduce the soil microbial biomass (Brookes and McGrath, 1984) and (Chander et al., 1995) and the activity of various microbial enzymes (Kandeler et al., 1996).

Several microbial cellular processes, such as synthesis of secondary metabolites (Weinberg, 1990), and mechanisms associated with the infection and virulence of microorganisms (Campoy et al., 2002; Davis et al., 2009) seem to be sensitive to zinc stress (Kunito et al., 2001) and zinc homeostasis (Hantke, 2001) and thus might be useful for evaluation of the toxic effects of zinc on microorganisms. Soil microbial communities hold a central place in terrestrial ecosystems, performing essential ecological processes or functions, such as nutrient cycling, plant nutrition or disease suppression (Bardgett et al., 2008). Therefore, it is useful to understand the consequences of zinc application on diversity and structure of microbial functional genes in soil ecosystems.

Different methods and approaches have been used to study the functional diversity of soil microbial community. Biolog EcoPlate, a technique based on levels of activities on various substrates, was used in different studies to evaluate the metabolic profile of soil microbial communities (Pająk et al., 2016; Tosi et al., 2016; Kelly et al., 1999; Zak et al., 1994) and determine the relative zinc sensitivity on the metabolic activities of the isolated microorganisms (Lock and Janssen, 2005). A range of novel methods, most of which are based on rRNA and rDNA analyses, have been developed to assess the functional diversity and structure of microbial communities. Polymerase chain reaction (PCR)-based fingerprinting, such as terminal restriction fragment length polymorphism (T-RFLP), has been used to analyze changes in diversity and structure of soil microbial community based on specific functional genes (Bannert et al., 2011). Quantitative real-time PCR is useful to analyze differences in the abundance of microbial function-specific genes (Lammel et al., 2015). However, there are some problems and biases in the PCR amplification step; therefore, these methods cannot be used as definite indicators of richness of ecological communities. Shotgun metagenomic sequencing and gene expression array analysis constitute an alternative approach to taxonomic and functional characterization of soil microbial community that avoids these limitations. The GeoChip-based functional gene array method simultaneously monitors the expression levels of thousands of genes (He et al., 2007). Such information is very useful in functional diversity studies to track highly expressed genes and genes critical in biogeochemical pathways. It is also important to obtain information on the microbial community response to environmental changes and factors that regulate diversity (Cong et al., 2015; Li et al., 2014).

Considering the potential increases in sugarcane productivity provided by zinc application in natural low-fertility soils worldwide, it is necessary to obtain a better understanding of the effects of zinc application on specialized functioning of the soil performed by microbial groups. For this purpose, we applied a functional gene microarray (GeoChip v. 5.0) (*i*) to assess the effects of zinc application on richness, diversity, evenness and structure of microbial functional genes in sugarcane-cultivated soil and (*ii*) to identify the response thresholds in abundance for groups of microbial functional genes to the zinc concentration added to the sugarcane-cultivated soil.

2. Materials and methods

2.1. Field experiment

Regional-scale field experiments were performed with RB867515 variety of sugarcane at the two major producing regions of sugarcane in the State of São Paulo, Brazil: Serra Azul (latitude 21°18′41″ S and longitude 47°33′59″W) and Assis (latitude 22°39′40″ S and longitude 50°23′58″ W) municipalities. The climate in the region is humid subtropical. Oxisol and Ultisol are the soil types at the Serra Azul and Assis municipalities, respectively. The field experiments at both municipalities were characterized by sandy soil with low natural fertility and available zinc concentrations of less than 0.5 mg dm^{-3} (Mellis et al., 2016). Four treatments with three replicate plots each were used in a completely randomized design in each location. Each experimental plot (75 m^2) consisted of five neighboring rows, each 10 m long and 1.4 m apart. Zinc was applied in the form of zinc sulfate ($ZnSO_4$) in the furrow at three different doses (5, 10 and 20 kg ha^{-1}) once at the beginning of the experiment on March 2011. The control treatment consisted of soil without application of zinc. In addition, fertilization was performed at sugarcane planting and consisted of 25 kg nitrogen (N) in the form of urea, 150 kg P_2O_5 (triple superphosphate), 100 kg K_2O (potassium oxide) and 3 kg of sulfur (S) per hectare. Broadcast fertilization was performed six months after planting (September 2011) with 27 kg N and 40.5 kg K_2O per hectare. In addition, 125 kg N and 100 kg K_2O per hectare were applied in three subsequent cycles of sugarcane ratoon.

2.2. Soil sampling

Soil samples were collected from each of three treatment and control soils in both sugarcane field experiments (4 treatments × 3 soil samples per treatment × 2 experimental fields = 24 soil samples) after the third sugarcane ratoon. Soil sampling consisted of five points in the furrow for each experimental plot: one central sampling point and four other sampling points at least 2.8 m apart from the central point and directed towards the four cardinal points. The soil cores were taken from the 0 to 40 cm topsoil layer using a 5-cm diameter aseptic cylindrical core. Each soil sample consisted of a composite of five soil cores collected within each experimental plot. Soil samples for chemical determination were immediately processed. Aliquots of soil samples were transported to the laboratory on ice and stored at −20 °C until further processing within 72 h after sampling.

2.3. Soil chemical measurements

The chemical measurements of each of the 24 soil samples were determined according to EMBRAPA (2011). Soil pH was measured on a soil/0.01 M $CaCl_2$ (1:5) suspension. Aluminum (Al), calcium (Ca), and magnesium (Mg) were extracted with 1 M potassium chloride. Ca and Mg were determined by atomic absorption spectrometry, whereas Al was determined by acid-base titration. Available phosphorous (P) and potassium (K) were extracted by ion-exchange resin and determined by colorimetry and atomic emission spectroscopy, respectively. Combined results were used for calculation of exchangeable bases (SB) as the sum of Ca, Mg, and K; cation-exchange capacity (CEC) as the sum of Ca, Mg, K, Al, and H; base saturation (V) as the percent relation between SB and CEC;