



Metagenomic analysis reveals microbial functional redundancies and specificities in a soil under different tillage and crop-management regimes



Renata Carolini Souza^{a,b}, Mariangela Hungria^{a,*}, Maurício Egídio Cantão^c, Ana Tereza Ribeiro Vasconcelos^d, Marco Antonio Nogueira^a, Vânia Aparecida Vicente^b

^a Embrapa Soja, Cx. Postal 231, 86001-970 Londrina, Paraná, Brazil

^b Universidade Federal do Paraná, Department Microbiology, Centro Politécnico, 81540-970 Curitiba, Paraná, Brazil

^c Embrapa Suínos e Aves, Cx. Postal 21, 89700-000 Concórdia, Santa Catarina, Brazil

^d Laboratório Nacional de Computação Científica, Rua Getúlio Vargas 333, 25651-071 Petrópolis, Rio de Janeiro, Brazil

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ABSTRACT

Information about microbial functionality in agricultural soils is still scarce, and in this study we used a shotgun metagenomic approach to compare different soil [conventional tillage (CT) with plowing and disking, and no-tillage (NT) with direct sowing into the residues of previous crops], and crop [crop succession (CS, soybean-summer/wheat-winter) or rotation (CR, soybean/maize-summer)/wheat/lupine/oat-winter] managements in a 13-year-old field experiment in southern Brazil. Differences were detected between NT and CT in some functional subsystems, e.g., NT had more sequences associated with the metabolism of aromatic compounds, which might be related to higher capacity to degrade pesticides, more sequences of the adenylate cyclase (cAMP) pathway, which might confer stability to the microbial community, among others. On the other hand, CT showed more sequences related to carbohydrate metabolism, what could be related with a lower content of organic matter and need to metabolize a broader range of carbon sources. Also, we detected differences related to crop management, e.g., crop rotation showed more sequences in the metabolism of amino acids and derivatives and carbohydrate subsystems, what might result from higher diversity of crop-residues added to the soil. However, it was notable that the differences in the diversity of taxa previously shown in the same experiment was far greater than the functional diversity reported now, emphasizing a high level of microbial functional redundancy.

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1. Introduction

Brazil's economy is strongly based on agriculture and, in 2013/2014, 55.2 million hectares were cultivated for grain production under different soil- and crop-management systems (CONAB, 2014). The conventional tillage (CT) system consists of the traditional practices of plowing and disking before sowing, and degradation of large areas worldwide has been attributed to these mechanical practices. Contrarily, the no-tillage (NT) system consists of sowing directly into the residues of the previous crop,

benefiting soil chemical, physical and biological properties, when compared with CT (Boddey et al., 2010; Calegari et al., 2008; Castro Filho et al., 2002; Derpsch, 1991; Lal et al., 2007; Silva et al., 2014). The first trials with NT in Brazil date from the 1970s; it is now practiced on more than 32 million hectares (FEBRAPDP, 2014). NT has marked benefits on soil microorganisms, in terms of microbial biomass and metabolic quotient (Babujsia et al., 2010; Balota et al., 1998, 2004; Bernard and Habash, 2009; Franchini et al., 2007; Hungria et al., 2009; Silva et al., 2010, 2014), and also in terms of functional groups, such as nitrogen-fixing rhizobia (Ferreira et al., 2000; Hungria and Vargas, 2000; Kaschuk et al., 2006; Pereira et al., 2007; Souza et al., 2013).

Another important practice for sustainability of productivity is crop rotation (CR). Although monoculture and crop succession (CS) with only two crops occupies most of the agricultural land globally, the adoption of CR with a minimum of three crops is a goal of agricultural conservationists (FAO, 2012). Benefits for soil chemical and physical properties have been reported in Brazil with the

* Corresponding author. Tel.: +55 4333716206; fax: +55 43 33716100.

E-mail addresses: renata.kerolini@hotmail.com (R.C. Souza),

mariangela.hungria@embrapa.br, hungria@cnpq.br,

biotecnologia.solo@hotmail.com (M. Hungria), mauricio.cantao@embrapa.br

(M.E. Cantão), atrv@lnccl.br (A.T.R. Vasconcelos), marco.nogueira@embrapa.br

(M.A. Nogueira), vaniava63@gmail.com (V.A. Vicente).

adoption of CR, in terms of increased carbon sequestration and organic matter (Babujia et al., 2010; Boddey et al., 2010; Calegari et al., 2008; Silva et al., 2014). However, it is noteworthy that detecting benefits in microbial biomass-related parameters due to the adoption of CR in comparison with CS is more difficult than in the comparison of NT and CT (Balota et al., 1998; Franchini et al., 2007; Hungria et al., 2009; Silva et al., 2010, 2014). It is also intriguing that in the studies performed in Brazil it has been difficult to detect changes in microbial diversity in the comparison of CS and CR, evaluated by the analysis of profiles of 16S rDNA-DGGE (Silva et al., 2013) or by metagenomics (Souza et al., 2013). Worldwide, other studies have also reported benefits especially in the comparison of NT over CT, e.g., by using BIOLOGTM and other microbial parameters in areas cropped with wheat (Lupwayi et al., 1998), maize (Govaerts et al., 2007) and barley (Lupwayi et al., 2001), among others.

Studies of soil metagenomics are increasing worldwide (e.g., Acosta-Martínez et al., 2010; Delmont et al., 2012; Fierer et al., 2007, 2012a,b), but in agricultural tropical soils are still scarce. Estimates are that Brazil hosts approximately 20% of the world's biological diversity, but the degree to which this applies to microbial diversity is still largely unknown (Pylro et al., 2014). Nevertheless, maintenance of ecosystem dynamics and land-use sustainability demand knowledge about microbial diversity and functionality. Previously, by using a shotgun-sequencing approach in soils of a long-term field experiment—13 years—under NT and CT with CS or CR, we revealed major differences in microbial diversity associated with tillage systems and, to a lesser extent, to crop management (Souza et al., 2013).

It is very important to establish linkages between soil microbial diversity and functionality, as our knowledge about this linkage, especially in tropical soils is still far from being clarified. Our hypothesis in this study is that soil microbial functionality might have a buffering capacity aiming at minimizing the impact of agricultural practices. Investigating soil management systems with higher (conventional tillage, monocultures) or lower (no-tillage, crop rotation) impacts might help to indicate to which extent soil microbes may preserve soil functioning.

2. Materials and methods

2.1. Description of the field trial and soil sampling and processing

The study was performed in a 13-year-old field trial established at the experimental station of Embrapa Soja, in Londrina, state of Paraná, Brazil, latitude 23°11'S, longitude 51°11'W, and elevation of 620 m. The soil is classified as Latossolo Vermelho Eutroférico (Brazilian system), rhodic eutrodox (US taxonomy). Information about climate conditions (Silva et al., 2010) and chemical and physical properties (Souza et al., 2013) has been given before; however, to facilitate the discussion, information about chemical and physical properties is also given in Supplementary Table S1.

The treatments consisted of conventional tillage (CT, with the traditional practices of soil plowing and disking), and no-tillage (NT, with direct sowing into the residues of the previous crop), each under crop succession (CS) [soybean (*Glycine max* L. Merr.) in the summer and wheat (*Triticum aestivum* L.) in the winter], or crop rotation (CR) [soybean and maize (*Zea mays* L.) in the summer and wheat, lupine (*Lupinus angustifolius* L.) and oat (*Avena strigosa* Schreb.) in the winter]. Crops grown in succession and rotation for the last 7 years were listed before (Souza et al., 2013). The four treatments are designated as NTS, NTR, CTS and CTR. Each plot measured 8 m width × 15 m length; the trial had a completely randomized block design, with four replicates.

Sampling was performed in early November of 2010, when the experiment was 13-years old, immediately before sowing of soybean (summer crop), and three weeks after harvesting the winter crop, wheat. Wheat residues have been incorporated to the soil in the CT and left on soil surface in the NT system.

Soil samples were taken from an area of 0.4 m² of the superficial layer (0–10 cm), with eight replicates per each of the four replicates of each treatment. A detailed description of sampling procedure and processing was given before (Souza et al., 2013).

2.2. DNA extraction, shotgun sequencing and sequences analyses

In the laboratory, plant residues were removed, soil samples were homogenized and passed through a 2-mm sieve before analysis. Metagenomic DNA was extracted by using 10 g of each soil replicate and the PowerMaxTM Soil DNA Isolation Kit (Mo Bio Laboratories) and submitted to sequencing analysis in the 454 platform (GS-FLX Titanium Roche Applied Science) at the Labinfo of LNCC (Petrópolis, Rio de Janeiro, Brazil, <http://www.labinfo.br>). For the pyrosequencing, DNA was randomly fragmented by nebulization with compressed nitrogen gas. Fragments of 300–800 bp were selected and submitted to sequence analysis with the Titanium kit, and the support PicoTiterPlate (Roche Applied Science) (Imelfort and Edwards, 2009). Replicates (Gomez-Alvarez et al., 2009) and Lucy Software (Chou and Holmes, 2001) were carried out to remove artificial duplicate reads (ADRs) and low-quality sequences. The data set was deposited in the NCBI-SRA (Sequence Read Archive, <http://www.ncbi.nlm.nih.gov/sra/?term=SRA050780>) and at the DDBJ database (<http://trace.ddbj.nig.ac.jp/DRAsearch/submission?acc=SRA050780>), with the submission accession number SRA050780. In this study functional data were analyzed and normalized by using the MG-RAST tools (Meyer et al., 2008).

2.3. Functional analyses of the sequences with SEED and KEGG

The sequences obtained were submitted to the MG-RAST (the Metagenomics RAST – <http://metagenomics.anl.gov>), and compared against SEED database for the functional classification in subsystems (Overbeek et al., 2005). According to the SEED website (http://theseed.org/wiki/Home_of_the_SEED), a subsystem represents a set of functional roles that make up a metabolic pathway, a complex, or a class of proteins. The levels of subsystems considered in SEED are: (1) highest level; (2) second highest level; (3) similar to a KEGG pathway; (4) actual functional assignment to the feature in question. Data were also compared against KEGG (Kyoto encyclopedia of genes and genomes) database (Kanehisa et al., 2004); KEGG is a database resource that integrates genomic, chemical and systemic functional information (<http://www.genome.jp/kegg/kegg1.html>). For the BLAST (basic local alignment search tool, National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>) search, a cut-off of minimum identity of 60% and *E*-value of 1×10^{-5} were considered.

Due to the agricultural interest in C and N metabolism under NT and CT, the sequences were also analyzed for the metabolic profile towards the main processes related to C and N metabolism, based on MG-RAST with the KEGG map.

2.4. Statistical analysis

Data from MG-RAST were submitted to statistical analysis with the STAMP (statistical analysis of metabolic profile) software (Parks and Beiko, 2010), to evaluate statistical differences in the metabolic profiles of the metagenomes considering all combinations in pairs. Statistical significance was estimated with the

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