Soil Biology & Biochemistry 115 (2017) 423–432

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Agricultural land use determines functional genetic diversity of soil microbial communities

Lokeshwaran Manoharan [a, *], Sandeep K. Kushwaha [a, b, c], Dag Ahrén [a, b], Katarina Hedlund [a]

[a] Department of Biology, Lund University, SE-223 62, Lund, Sweden
[b] National Bioinformatics Infrastructure Sweden (NBIS), Department of Biology, Lund University, Lund, Sweden
[c] Department of Plant Breeding, Swedish University of Agriculture Sciences, Alnarp, Sweden

ARTICLE INFO

Article history:
Received 21 March 2017
Received in revised form
2 August 2017
Accepted 10 September 2017
Available online 20 September 2017

Keywords:
Microbial ecology
Functional genes
Captured metagenomics
Land-use management
MiSeq paired-end sequencing

ABSTRACT

Microbial communities play a major role in the degradation of soil organic matter (SOM) in soils. Despite its significance, the functional diversity of the highly diverse microbial communities is poorly understood. To address this, we applied a recently developed technique, captured metagenomics, to determine the effects of land-use on the functional genetic diversity of genes involved in the carbon degradation of SOM in five pairs of agricultural soils with either winter wheat or grass as management. In addition, 16S rRNA based amplicon sequencing was used to study the taxonomic composition in the same soils. The functional genes resulting from the captured metagenomes had a higher abundance and diversity of sequences coding for enzymes degrading SOM in the grasslands compared to the wheat soils. Though the taxonomic diversity did not correlate with the land use. Amounts of C and N (organic matter content) in the soils affected both functional and taxonomic diversity of the microbial communities, where N was highly correlated to their functions and C was highly correlated to their taxonomy. Captured metagenomic analyses of the functional genes may provide a measure of the potential SOM degradation capacity by soil microbial communities at a high resolution. This can be used for assessments of how agricultural management affects the functioning of soil communities.

1. Introduction

Soil microbial communities play an important role in many ecosystem functions such as nutrient cycling, leading to soil fertility and plant productivity (Doran and Zeiss, 2000; Schimel and Schaeffer, 2012a; Nie et al., 2015). Ecosystem functions in soils largely depend on the activity and complexity of the microbial communities that are influenced by multiple physical, chemical and biological aspects of the soil ecosystem (Fierer et al., 2012). Different land-use can alter microbial communities in soils thereby changing a number of soil ecosystem functions (de Vries et al., 2013) including C cycling (Mackelprang et al., 2011; Schimel and Schaeffer, 2012b). The microbial contribution to carbon and nutrient cycling is highly complex, as management of soils may have both direct and indirect effects on their community structure and functions (Bardgett et al., 2008; Singh et al., 2010).

Effects of land-use on ecosystem functions (Ehrenfeld et al., 2005) are often studied with focus on microbial community composition (Araujo et al., 2013; Garcia-Franco et al., 2015). Microbial community composition has often been related to different types of ecosystems (Mackelprang et al., 2011; Delmont et al., 2012) and their effect on ecosystem functioning (Dunbar et al., 1999; Yeager et al., 2004). Though, it has been argued that the taxonomic information of the microbial communities on its own is often not powerful enough to predict their functional capabilities (Barberan et al., 2012). Thus by quantifying functional diversity of microbial communities, we may answer questions on what the microbial communities do, how they are affected by management and how this influence a number of soil functions (Fierer et al., 2014).

Recently it has been suggested that studying functional genes and community functional composition would be a better predictor of soil functions (Gravel et al., 2012; Yang et al., 2014). The knowledge on functional gene composition of related enzymes and

* Corresponding author. Present address: Department of Ecogenomics and Systems Biology, University of Vienna, A-1090, Vienna, Austria.
E-mail address: lokesh.cbt@gmail.com (L. Manoharan).

http://dx.doi.org/10.1016/j.soilbio.2017.09.011
0038-0717/

their diversity, rather than using taxonomic patterns, can be a potential key in understanding cycling of organic matter in soils (Gougoulias et al., 2014; Yang et al., 2014). To achieve information on the diversity of functional genes and thus the potential of functions of different soil microbial communities, whole metagenomic sequencing of soil microbial communities has been tested (Dinsdale et al., 2008; Delmont et al., 2012). Though, even with the advancements in the field of sequencing technology, metagenomics require large computational resources to deal with high amounts of data to achieve a reasonable resolution (Escobar-Zepeda et al., 2015). A recent development of a technique called 'captured metagenomics' can target a high diversity of selected functional genes and may help in obtaining information in a high resolution to reveal functional potentials of microbial communities (Manoharan et al., 2015). This method allows for a deeper understanding of how land-use affects the functional potential of microbial communities in soil to degrade organic matter by targeting the related genes in C cycling. The enzymes responsible for degradation and transformation of various complex C molecules such as cellulose and hemicellulose in soil represent a key factor in C cycling (Eichorst and Kuske, 2012). Similarly, proteases that are excreted by the microbial communities are mainly responsible for the N mineralization and their cycling (Vranova et al., 2013). The 'captured metagenomics' technique, which was applied in this study, is based on the available information of gene sequences that produce enzymes involved in the C and N cycling in soils, such as glyosyl hydrolases from the CAZy database (Cantarel et al., 2009) and proteases from the MEROPS database (Rawlings et al., 2002). In this study, more than 300,000 functional genes (from the above mentioned databases) have been targeted using more than 400,000 unique 50-mer oligonucleotide probes (Kushwaha et al., 2015; Manoharan et al., 2015).

Here, we address the impact of agricultural land-use on the diversity of soil microbial communities, in order to first determine the functional genetic diversity by applying captured metagenomics on genes encoding for enzymes related to organic matter degradation. Second, the taxonomic diversity of bacterial communities will be determined by applying 16S amplicon sequencing. We have applied both methods on agricultural soils that are under conventional farming practices with winter wheat or permanent grasslands to test the following questions on land-use management of soils. (1) Does agricultural land use affect the functional genetic diversity of microbial communities; (2) Does agricultural land-use affect the taxonomic composition of microbial communities; (3) What soil properties can are related to the functional and taxonomical patterns of the microbial communities respectively.

2. Materials and methods

2.1. Sampling sites and DNA extraction

The soils that were used in this study came from 5 farms located in the south of Sweden that were part of the EU based project SOILSERVICE (Tsiafouli et al., 2015). Each farm had fields with winter wheat (W) or grasslands (G) that were in close proximity to each other. The soil properties and location of these soil sites are presented in the Supplementary Table S1. Multiple soil cores (0–15 cm depth) were taken from each field and pooled together in October 2012. The soils were sieved (2.5 mm) and stored at −20 °C, later kept at 4 °C upon DNA extraction. The DNA extractions were carried out using the Nucleospin soil DNA isolation kit (Machery & Nagel, Germany) according to their protocol with a starting material of 0.5 g from soil. Multiple extractions were carried out for each soil and the extraction with the best quality and quantity (NanoDrop Technologies, USA) was chosen for further analysis.

2.2. 16S amplicon library preparation

The taxonomic compositions of the soil samples were primarily obtained through sequencing of the PCR amplicons of the bacterial ribosomal small subunit (16S). The amplicon libraries were prepared by designing the PCR primers for V3-V4 region along with the adaptors for 454 sequencing (Herlemann et al., 2011). The forward primer B341F [5′-CCTACGGGNGGCWGCAG-3′] was designed with 454-adaptor-A and then the reverse primer B805R [5′-GACTACHVGGGTATCTAATCC-3′] was designed with a multiplexing identifier (MID) including 454-adaptor-B. The PCR parameters and reagents were optimized according to the DNA polymerase Phire Hot Start (Thermo Fisher Scientific Inc., USA). For each soil DNA sample, three 25 μl (5 μl-5X Buffer; 0.5 μl-10 mM dNTPs; 1 μl-10 μM forward primer; 2μl-5μM reverse primer; 0.5 μl-DNA polymerase; 0.5 μl-Bovine serum albumin; 2.5 μl-Template DNA; 13 μl-MilliQ water) PCR reactions were carried out. The three PCR amplification (Initial denaturation step at 98 °C for 30s; 27 cycles of denaturation at 98 °C for 5s, annealing at 56 °C for 5s, extension at 72 °C for 10s; final extension at 72 °C for 60s and refrigeration at 4 °C) products for each sample were then pooled together. These products were purified using QIAquick PCR purification kit (Qiagen, The Netherlands) and then quantified using Quant-it Pico Green kit (Invitrogen, USA).

2.3. Sequence capture

The metagenomic DNA obtained from the soil samples was processed to enrich for fragments of interest through the method of so called 'captured metagenomics' using oligonucleotide probes according to Kushwaha et al. (2015) and Manoharan et al. (2015). The soil DNA libraries were prepared through random fragmentation of the DNA to around 300–350bp, which are then ligated with indexed (sample specific) adaptors optimized for sequencing in Illumina platform. The quality and quantity of the DNA libraries were monitored at multiple steps throughout the experiment. These soil DNA libraries were then briefly amplified (ligation mediated PCR) and pooled together according to the amounts recommended by the SeqCap EZ protocol (Roche NimbleGen, USA). In total, 1 μg of DNA library pool was obtained from equal amounts of DNA libraries from different samples. This DNA library pool is then mixed for hybridization with oligonucleotide probes (Roche NimbleGen, USA) designed to target genes coding for enzymes related to organic matter degradation such as carbohydrate-active enzymes (CAZy) and proteases (MEROPS) along with hybridization enhancing oligos. This mixture was incubated at 47 °C for 72 h and the mix is subsequently washed with buffers according to the manufacturer (SeqCap EZ: Roche NimbleGen, USA) at 47 °C. The washing steps include streptavidin dynabeads and a magnetic device to remove unbound DNA fragments also at 47 °C. The bound DNA (captured) was then briefly amplified (ligation mediated PCR) before they are purified and quantified. The DNA libraries for the 10 soil samples were prepared, 'captured' and sequenced at the service facility Ambry Genetics (USA).

2.4. Sequencing

The purified 16S amplicons were pooled in equal amounts (as each amplicon had a sample specific 454 MID) and sequenced on a ¼ region of the 454 plate using a GS FLX Titanium series with Lib-A chemistry optimized for amplicons in 454 pyrosequencing at the sequencing facility at Lund University (Sweden). With the samples from the sequence capture, DNA libraries are already (sample-specific) indexed in their adaptors when are pooled and captured. The captured pool would have DNA fragments along with their