Impact of crop diversification and low-input farming on soil microbial diversity
Impacto de la diversificación de cultıvos y prácticas de bajo impacto sobre la diversidad microbiológica

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Abstract

Microbial community existing in soil ecosystems is a major indicator of soil quality. Microorganisms in soil provide and/or contribute major biogeochemical cycles of carbon and nitrogen. They shape soil characteristics physically and chemically along with providing for Nitrogen, Sulphur, Phosphate and other nutritional cycles in agricultural areas locally. This project focuses on determination of soil microbiome community as the types of species existing and the abundance of each species through next-generation sequencing of 16S ribosomal metagenomic DNA from different agricultural areas. The overall data will provide a vast range of information of soil microbial community structure and the effect of different agricultural practices on community structure.

Keywords: Metagenomics; soil microbiome; next-generation sequencing; 16S ribosomal DNA.

Resumen

Las comunidades microbianas existen en los ecosistemas de suelo y son indicadoras de su calidad. Los microorganismos en el suelo, proveen y contribuyen a los grandes ciclos geoquímicos del carbono y nitrógeno. Determinan las características físico-químicas y proveen de forma localizada de nitrógeno, sulfuro, fostato en la agricultura. El presente proyecto enfoca la determinación de comunidades microbiológicas, incluyendo especies y abundancia por medio de secuenciación de nueva generación de ADN de diferentes áreas agrícolas. Los datos generados van a dar una información muy amplia sobre las comunidades microbianas del suelo y el impacto de diferentes prácticas agrícolas sobre su estructura.

Palabras clave: Metagenómica, microbioma del suelo, secuenciación de nueva generación, 16S.

1. INTRODUCTION

Soil quality has been defined as “the capacity of a soil to function, within the limits imposed by the ecosystem, to preserve the biological productivity and environmental quality, and promote plant, animal and human health” [1]. Microorganisms living in soil are abundant and highly diverse. These microorganisms are the key players of many soil functions such as biogeochemical cycling, plant productivity or climate regulation and are essential for the integrity of terrestrial ecosystems. The environmental pressure on soil are often related to human activities and diminishingly decreasing the overall quality and sustainability of soil in both agricultural and forest ecosystems [2]–[4].

Soils represent the most diverse and important ecosystem on the planet. Most of the biodiversity of agroecosystems found in the soil, and the functions performed by soil biota have considerable direct and indirect effects on crop growth and quality, nutrient cycle quality and the sustainability of soil productivity [5], [6]. Soil quality is considered as an integrative indicator of environmental quality, food security and economic viability. The living community of soils, especially the microbial community, is evaluated as high importance in sustainable agricultural production and the conservation of major ecological cycles. Therefore, soil is accepted as an indicator for monitoring land management and quality assessments in relation with agricultural practices. The use of microbial community structure and diversity as an indicator to monitor soil quality is both challenging due to lack of understanding of community structure and soil function, and very promising due to direct correlation of major nutrient cycles related to agricultural processes and soil microbial community [7], [8]. Soil biota also contributes directly and indirectly to the resistance and resilience of agroecosystems to abiotic disturbances and stress factors such as nutritional chemicals, anthropogenic, climatological and hydrological disturbances. Microbial community present in the soil are the most sensitive and rapid indicators of perturbations and land use changes. In this manner, quantitative identification of microbial community structure and naturally the diversity of microbiota has become great interest as one of the strongest tools for soil quality evaluation [9]–[11]

Agricultural land management is one of the most significant activity to alter soil characteristics in physical, chemical, and biological properties. This fact is particularly relevant in regions such as Mediterranean, where climate with land management can easily lead increased rates of erosion and other degradation processes of agricultural land due to arid seasons etc. These issues may can lead to loss of soil fertility and reduction in the diversity and abundance of soil microorganisms [12]–[14]. Agricultural practices alter the soil microbial community in many ways. Chemical treatments through pesticides and herbicides along with tillage are some of the negative effects on soil microbial community structure and function. On the other hand, some organic fertilisers and other repeated application of manures may lead increased soil microbial community function [14]–[18].

Given the crucial importance of diversity and abundance of soil microbiota, there are several techniques to assess community structure profile. One of the most effective technique rely on DNA sequencing technology where specific amplicons selected within the bacterial genome and sequenced through high-throughput next-generation sequencing to identify and quantify bacterial community in metagenomic samples. Sequencing 16S ribosomal DNA of metagenomic soil samples considered as the ultimate assay to obtain community structure data in soils. Different DNA fragments within 16S ribosomal DNA of bacteria are hypervariable regions meaning that the specific sequences are greatly vary through species and it allows the differentiation between different species in soil and their abundancy through sequencing data and bioinformatics analysis of data [19]–[21].

2. MATERIALS AND METHODS

Quantitative and qualitative analysis of soil microbial communitytructure will be performed in two different experimental set-up as following:

- Above-ground (agricultural products) biodiversity is increased by planting vetch and barley between mandarin trees to evaluate how the increased biodiversity of agricultural products affects below-ground biodiversity (microorganisms present in soil).
- Long term practices such as conventional farms, organic farms and biodynamic farms has been handled in specific farms in Murcia/Spain. The effect of different agricultural practices will be influencing the soil microbial biodiversity and abundance. The samples from different farms will be analysed to conclude the overall effect.

2.1. Sampling

The samples were collected from 0-10 cm from the soil surface and immediately put on ice to be transferred to -20°C to be stored until DNA extraction.

2.2. DNA extraction

The genomic DNA from soil samples will be extracted by using DNeasy PowerSoil Kit (QIAGEN)

2.3. Target Selection

The amplification of 16S ribosomal DNA regions will be carries out by using commercial kit Ion 16S™ Metagenomics Kit (ThermoFisher). The process will yield amplicons which will be used to identify microbial community based on sequencing data.

2.4. Screening of Amplicons

Identification of isolated and selected DNA sample will be performed by using Agilent 2100 Bioanalyzer system in competence with Agilent High Sensitivity DNA kit and suitable chips. The analysis allows to differentiating different DNA samples by size and calculating their concentrations.

2.5. Library construction

The library construction is done to anneal sequencing adaptors to DNA samples along with a barcoding process. DNA barcodes allows pooling samples in a single chip to be sequenced to be identified further in bioinformatics studies according to specific barcode sequences embedded to each sample. Library preparation will be carried out by using Ion Xpress Plus Fragment Library Kit. DNA barcoding will be carried out by using Ion Xpress™ Barcode Adapters 1–16.

2.6. Template preparation

The templates are the ultimate clonally amplified DNA samples to be sequenced. The system works as an emulsion PCR method for clonal amplification of prepared library by using Ion OneTouch™ 2 instrument and suitable Ion PGM™ Hi-Q View OT2 Kit.

2.7. Sequencing

Sequencing process will be carried out by using Ion Torrent PGM™ (ThermoFisher) by using suitable Ion PGM™ Hi-Q View Sequencing Kit and Ion 314™ Chip kit.

2.8. Physical and chemical soil properties

Fluorometric microplate method for enzyme assays and conventional physical and chemical soil properties by a common procedure to be delivered as Handbook in the Diverfarming project (www.diverfarming.eu), where this thesis is implemented.

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