Impact of different crop rotation sequences and management practices on soil bacterial diversity in Northern Netherlands

Impacto de diferentes secuencias de rotación de cultivo y prácticas de manejo en la diversidad bacteriana edáfica en Holanda Septentrional

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Abstract

Identification of soil bacterial community structure and diversity provides a huge insight into soil quality due to the intense biochemical relationships between soil microbiome and interacting plants. The aim of this study was to assess how different diversified cropping systems under two different management (conventional and biodynamic) can affect soil bacterial diversity. Soil bacterial community was identified through next-generation sequencing of 16S rRNA coding genes of soil bacteria. The findings showed that different sequences of crop rotations, period of implementation of rotations and management practice had a strong influence on soil microbial biomass, bacterial biodiversity and the abundance of different bacterial phyla, without a specific trend regulated by these factors.

Keywords: soil microbiome; bacterial community; 16S rRNA; next-generation sequencing; rotations.

Resumen

La identificación de la estructura y diversidad bacteriana edáfica proporciona una visión enorme de la calidad del suelo debido a las intensas relaciones bioquímicas entre el microbioma del suelo y las plantas que interactúan. El objetivo de este estudio fue evaluar cómo diferentes sistemas de cultivo diversificados bajo dos diferentes tipos de manejo (convencional y biodinámico) afectan a la diversidad bacteriana del suelo. La comunidad bacteriana del suelo se identificó a través de la secuenciación de próxima generación de genes de codificación del RNAr 16S bacteriano. Los resultados mostraron que diferentes secuencias de rotaciones de cultivos, el periodo de implementación de las rotaciones y la práctica de manejo tienen una fuerte influencia en la biomasa microbiana, la biodiversidad bacteriana y la abundancia de diferentes filos bacterianos.

Palabras clave: microbioma del suelo; comunidad bacteriana; RNAr16S; secuenciación de próxima generación; rotaciones.

1. INTRODUCTION

Soil microorganisms, especially bacteria, play a non-replaceable role in soil processes contributing to the nutrient cycling, degradation and decomposition of organic and inorganic matter [1], contributing to soil fertility and the regulation of biogeochemical cycles. Moreover, the amount of soil microbial biomass plays a major role in driving the balance between the release of soil carbon (respiration) and its sequestration in soil organic matter along with the cycle of free nitrogen ions in agricultural ecosystems.

Even though monocropping is the agricultural trend of the past decades, it brings several environmental and ecological issues such as decline of soil fertility and biodiversity loss [2]. Crop rotations for a given agricultural field is the simplest and yet very strong practice to keep soil healthy in many ways including the bacterial biodiversity [3]. Soil microbes, which are considered as a second genome of plants, play fundamental roles in plant growth and health [4]. It is also proven that the biodiversity in soil bacterial population is strongly related and can be manipulated by changing the above-ground biodiversity [4]. Thus, the aim of this research was to assess how different rotation sequences performed under two different management practices (conventional and organic) can influence soil bacterial diversity.

2. MATERIALS AND METHODS

2.1 Experimental setup

This study was performed in collaboration with different private farms in the cities Leeuwarden and Groningen, Northern Netherlands. We focused on six different farms with different diversified cropping systems based on different rotations, grown under two different managements, conventional and biodynamic, for ≥ 10 years. The diversified cropping systems (DCS) studied are:

DCS 1. Biodynamic: wheat-peas-grass-maize-grass rotation (rotation with addition of fertilizer)

DSC 2. Conventional: grass-maize-grass rotation

DSC 3. Biodynamic: grass glover-cauliflower-oat-potato-phacelia rotation (10 years system)

DSC 4. Biodynamic: grass clover-cauliflower-oat-potato-phacelia rotation (20 years system)

DSC 5. Conventional: potatoes-winter wheat-winter barley (rotation with addition of mycorrhiza and fertilizer)

DSC 6. Conventional: potatoes-winter wheat-winter barley (rotation with no addition of mycorrhiza)

2.2 Soil sampling and analyses

Soil was sampled after harvest in summer 2018 at 0-10 cm depth. Five random samples were taken per diversified cropping system. The samples were taken into ice with sterile bags in the field and carried to the laboratory, sieved <2 mm and kept at -20ºC until analyses. DNA extraction from the soil samples was carried out by using DNeasy PowerSoil Kit, QIAGEN. Assignments of purity and concentration values were done by using NanoDrop™ 2000/2000c Spectrophotometer and by using Qubit® 2.0 Fluorometer and corresponding high sensitivity assay kit, Qubit dsDNA HS Assay Kit, respectively. Amplification of 16S hypervariable regions was carried out by using, Ion 16S™ Metagenomics Kit, ThermoFisher Scientific. Agilent 2100 Bioanalyzer® instrument used to evaluate concentrations and purity with the suitable Agilent High Sensitivity DNA Kit. Library preparation process was carried out by using Ion Xpress™ Plus gDNA Fragment Library Preparation Kit, ThermoFisher Scientific. Sequencing adaptors were used by Ion Xpress™ Barcode Adapters kit, ThermoFisher Scientific. Prepared library amplicons were

processed for template preparation by using Ion Sphere™ Particles (ISPs) to create template-positive ISPs via Ion OneTouch™ 2 System with suitable Ion PGM™ Hi-Q™ View OT2 Kit, ThermoFisher followed by the enrichment process using Ion OneTouch™ ES. Sequencing was carried out by Ion PGM™ System, Ion PGM™ Torrent Server and suitable Ion PGM™ Hi-Q™ View Sequencing kit in competence with sequencing chips, Ion 314™ Chip v2 kit.

Raw sequencing results were processed through bioinformatics analyses to identify bacterial groups and families present in each sample. The process consisted of creating Operational Taxonomic Unit (OTU) tables by using the software QIIME™. Overall results were obtained by comparing the OTU tables against SILVA™ online database via QIIME.

3. RESULTS AND DISCUSSION

Microbial biomass, estimated by DNA content in soil, was 5059 ng g^{-1}, 26409 ng g^{-1}, 6600 ng g^{-1}, 5258 ng g^{-1}, 3111 ng g^{-1} and 2764 ng g^{-1} for DCS 1, 2, 3, 4, 5 and 6, respectively. Soil bacterial biodiversity (chao index) is shown in Figure 1. CS3 showed significantly highest biodiversity, followed by CS2 > CS1 > CS4 = CS6. DCS5 is not included in the figure 1 due to the low number of reads for the data set (samples are to be repeated). These results highlight that soil bacterial biomass and diversity in arable crops under Atlantic climate in the Netherlands is not related to management system. It is normally observed that conventional management has lower soil biodiversity than organic practices such as biodynamic due to the use of high amount of pesticides [5]. CS3 and CS4 have the same rotation sequence, but CS4 has been performed for longer. However, the longest cultivation (20 years) seemed to negatively affect bacterial diversity compared to 10 years of cultivation. Moreover, long rotation sequences were not related either with higher biomass and biodiversity, since CS2 with only grass-maize rotation contributed to higher biodiversity. Different types of fertilizers used can differently affect bacterial diversity. It has been usually reported that mineral fertilizers have long-term detrimental effects on soil biodiversity [6]. However, this study of farms managed for more than 10 years under the same system, showed that conventional management with mineral fertilizers is not always related to negative impacts on microbial biomass or bacterial diversity, contrary to previous findings.

Figure 2 shows the identified phyla in the studied DCS and their abundance. It is observed that Actinobacteria, Acidobacteria and Proteobacteria phyla tended to significantly vary among DCS. Bacterial phyla Saccharibacteria, Chloroflexi and Acidobacteria were significantly lower in abundance in the longer (20 years) rotation fields (DCS4) in comparison to 10 years rotation fields (DSC3). On the other hand, Actinobacteria, Chlamydiae and Firmicutes seem to be increased in with the longer rotation history. The addition of mycorrhiza tended to decrease the proportion of Actinobacteria and Bacteroidetes, while significantly increased the abundance of Acidobacteria, Chloroflexi and Proteobacteria.

4. CONCLUSIONS

Microbial biomass and bacterial biodiversity and structure significantly changed with different rotations and management practices, with no direct relationship with rotation history or management practice. Longer sequences, longer periods of rotations or biodynamic manager was not related with higher biodiversity, as initially expected. Actinobacteria, Proteobacteria, Bacterioidetes, Chloroflexi and Chlamydiae were the bacterial phyla most sensitive to changes in diversified cropping systems.

5. ACKKNOWLEDGEMENTS

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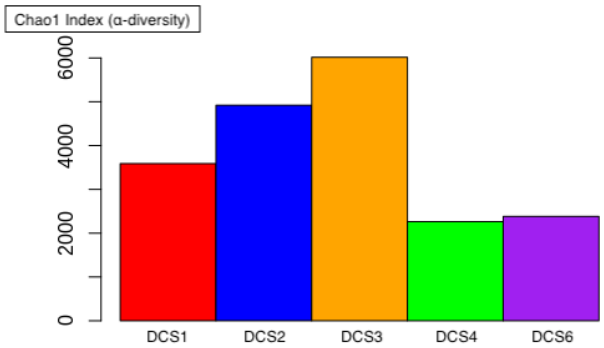

Figure 1. The α-diversity (Chao index) of the bacterial communities in different diversified cropping systems.

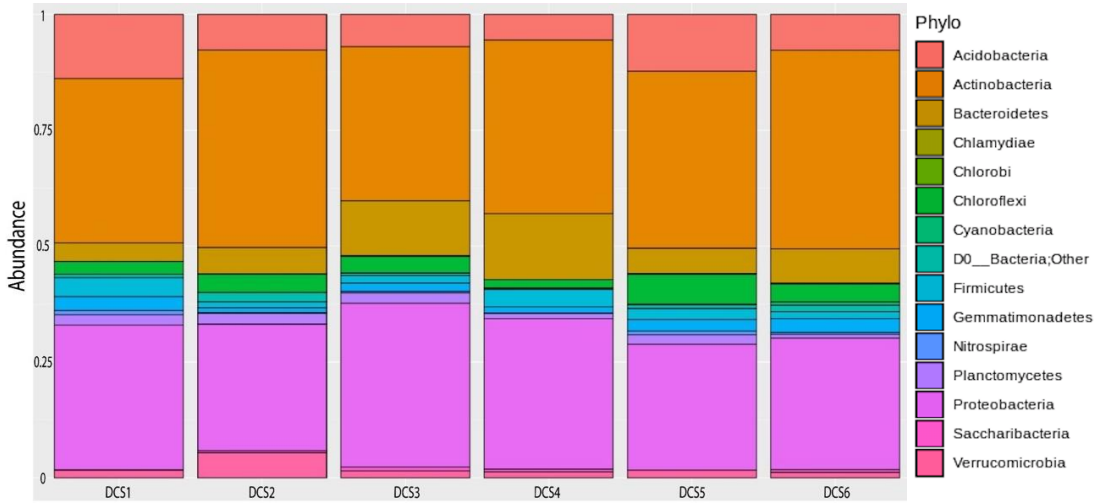

Figure 2. The relative abundance of bacteria at the phylum level across all soil samples. Each column represents a data set explained as in methods section (bacterial phylum abundance <1% was ignored).