

Long-term impact of sustainable land management practices on soil bacterial community in an almond orchard in south Spain

Impacto a largo plazo de prácticas de manejo sostenible del suelo en la comunidad bacteriana edáfica de un cultivo de almendros en el sur de España

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Abstract

A healthy bacterial community structure is one of the main aspects of soil quality indicators. Modern agricultural practices, where intense tillage and chemical fertilizers are applied, continue to put environmental balance and sustainability of production at stake day by day. In this study, we aimed to assess the long-term effect of low-input sustainable practices such as reduced tillage and green manure on soil bacterial communities in an almond orchard from south Spain. Reduced tillage and addition of green manure has shown the most stable bacterial community structure and species abundance with respect to conventional practice. Soil organic carbon and bacterial community are improved by low-input sustainable approach.

Keywords: 16S rRNA; soil bacteria; biodiversity; low-input farming; next generation sequencing.

Resumen

Una estructura de comunidad bacteriana saludable es uno de los principales indicadores de calidad del suelo. Las prácticas agrícolas modernas en las que se aplican labranza intensa y fertilizantes químicos continúan poniendo en juego el equilibrio ambiental y la sostenibilidad de la producción día a día. En este estudio, nuestro objetivo fue evaluar el efecto a largo plazo de prácticas sostenibles de bajos insumos, como la labranza reducida y el abono verde sobre la comunidad bacteriana edáfica en un cultivo de almendros del sur de España. La labranza reducida y la adición de abono verde han demostrado la estructura de la comunidad bacteriana más estable y la abundancia de especies con respecto a los métodos convencionales. El carbono orgánico del suelo y la comunidad bacteriana se mejoran con un enfoque sostenible de bajos insumos.

Palabras clave: 16S ARN; bacteria; biodiversidad; agricultura bajos insumos; secuenciación.

1. INTRODUCTION

Bacteria plays many crucial roles in soil dynamics such as regulation of macro biogeochemical cycles, nutrient cycling activities in terms of carbon and nitrogen ions along with degradation and decomposition of organic and inorganic matter (1). For any given crop field, the balance between soil carbon and free nitrogen ions are main aspects of healthy and productive

farming. Bacterial species regulate the availability and cycling of carbon and nitrogen ions in soil and bacterial population abundance and optimal biodiversity among species are one of the main soil quality indicators up to date (2).

Increasingly in the global scale, conventional way of agriculture has become highly mechanized and environmentally harmful. The non-sustainable practices have started to break the supply of key ecosystem services between crops and soil quality (3). Without these ecosystem services, modern agriculture has become dependent on off-farm inputs such as high tillage, chemical fertilizers which creates a negative loop for further biodiversity and soil quality reduction (4).

Sustainable land management practices such as reduced tillage and cover crops are proven to be contributing to prevent soil loss, organic carbon and other nutrient losses. Reduced tillage have long term positive effects on conserving soil quality and ecological and economical drawbacks of erosion and quality loss (5). Additionally by using reduced tillage and green manure systems, an increase of final product along with the necessity of fertilizers may be reduced/removed (3,5). Green manure application in soil is proven to improve hydraulic properties of the soil, increasing macroporosity and hydraulic conductivity, and elevating various aspects of soil quality (6).

The objective of this study was to assess the long term effect of low-input sustainable practices such as reduced tillage and green manure on soil bacterial communities in an almond orchard from south Spain.

2. MATERIALS AND METHODS

2.1 Experimental setup

The study area was located in an almond orchard (*Prunus dulcis*) in Alhagüeces, Region of Murcia (35° 57' 31''N, 0° 56' 17''W). Experimental area consists of 53 trees (0.05 ha) representing a total area of 1.40 ha. The area has 17.5 °C of mean annual temperature and 280 mm of mean annual precipitation. The experimental strategy was set up in 2009, where three different treatments were established in triplicate: i) almond monocrop with intense tillage (chisel ploughing 4-5 times yr⁻¹ at 20 cm) (AIT); ii) almond monocrop with reduced tillage (chisel ploughing 2 times yr⁻¹ at 20 cm) (ART); and iii) almond orchard with reduced tillage diversified with *Avena sativa* & *Vicia sativa*(1:3 at 150 kg ha⁻¹) applied to soil as green manure (AVV).

2.2 Soil sampling and analyses

Soil was sampled in November 2019 at 0-10 cm depth. Five composite random samples were collected per treatment. Samples were kept in cooler with ice using sterile bags and taken to the laboratory, sieved <2 mm and kept at -20 °C until DNA isolation. DNA extraction from soil 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 combined with 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 combined with Ion Xpress™ Barcode Adapters kit, ThermoFisher Scientific. Prepared library amplicons were processed for template preparation by using Ion Sphere™ Particles (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 316™ Chip v2 kit. Soil organic carbon (SOC) was determined by an elemental CHNS-O analyzer (EA-1108, Carlo Erba) with air-dried soil samples, sieved at < 2 mm and ground.

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. A One-way ANOVA with Tukey's post hoc at $P < 0.05$ was performed to assess differences among treatments. Principle co-ordinate analysis (PCoA) was conducted to assess the structure of bacterial community in the different treatments.

3. RESULTS AND DISCUSSION

Total DNA and SOC followed the same trend, with increasing values as AIT<ART<AAV, however, differences were not significant (Table 1). These results show the increasing trend in soil organic matter and the abundance of microorganisms when applying reduced tillage and addition of green manure, as previously reported in other studies (7–9). According to the bacterial Shannon index, which is considered an indicator of species diversity, no significant differences were observed among treatments (Fig. 1). So, the different long-term practices have not led to changes in soil bacterial biodiversity. The reason for this finding might be that although less intense, all systems causes soil disturbance by tillage, and so biodiversity remains almost intact (10,11). According to a PCoA analysis (Fig. 2), the composition of the bacterial community was different between all treatments. AAV and ART clustered closer, indicating that reduced tillage has selected a specific microbial community different to that present when tillage frequency is higher, and so, more adapted to perturbations. Thus, reduced tillage showed lower bacterial diversity than intense tillage, yet the community structure is more similar to that where green manure is used and the microbial abundance is higher. In addition, although no differences in Shannon index was detected, the bacterial community structure is different among treatments, suggesting that biodiversity indices may not be suitable to assess the efficiency of management practices in agricultural soils.

4. CONCLUSIONS

Reduced tillage and addition of green manure are increasing soil organic matter, associated to higher microbial abundance and changes in the bacterial community structure compared to the conventional almond monocrop with intense tillage. The use of *A. sativa* and *V. sativa* contributed to the highest soil organic matter, microbial abundance and bacterial diversity. Even though the biodiversity and organic carbon values are not significantly different among treatments, green manure addition is showing a positive trend to improve bacterial community stability and abundance.

5. ACKNOWLEDGEMENTS

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Table 1. Total DNA content and soil organic carbon (SOC) in the different treatments. Values are mean±standard error (n=5)

	AIT	ART	AAV	F-ANOVA
DNA (ng g⁻¹)	6012.31±4.20	6136.47±1.81	8756.14±6.57	0.025 not significant
SOC (g kg⁻¹)	17.28±0.81	18.12±0.26	19.08±0.92	0.058 not significant

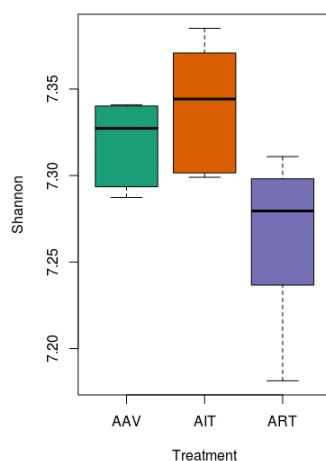


Figure 1. Shannon index of bacterial communities for each plot type prepared using the rarefied table with minimum number of reads and Shannon (vegan package) values.

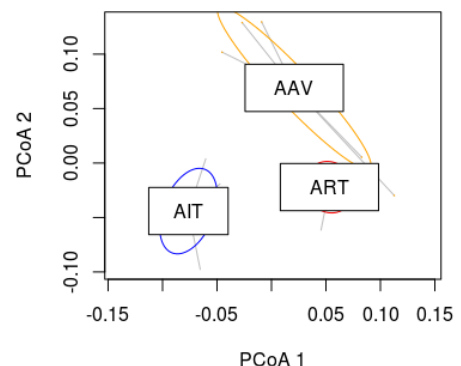


Figure 2. Principle co-ordinate analysis (PCoA) of bacterial communities by using bray method. Axis of PCoA 1 and PCoA 2 indicates bacterial abundance (numerical) and composition (coloured shapes).