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Soil microbial community responses to different sugarcane management strategies as revealed by 16S metagenomics

PD Fontana[1], L Orru[2], CA Fontana[1], PS Cocconcelli[3], GM Vignolo[4] and SM Salazar[1]

[1]INTA EEA Famaillá, Tucumán, Argentina; fontana.paola@inta.gob.ar
[2]Council for Agricultural Research and Economics (CREA) Research Centre for Genomics & Bioinformatics, Fiorenzuola d'Arda PC, Italy
[3]Dipartimento di Scienze e Tecnologie Alimentari per una filiera agro-alimentare Sostenibile (DISTAS), Università Cattolica del Sarco Cuore, Cremona-Piacenza, Italy
[4]CERELA-CONICET, Tucumán, Argentina

Abstract Sugarcane cultivation in Argentina is distributed in three geographic regions: Tucumán, Northern (Salta and Jujuy) and Littoral (Santa Fe and Misiones), covering about 376,223 ha. Tucumán has traditionally been the most important region with 68% of the total production. Since new agricultural techniques, such as green-cane harvesting and sugarcane crop rotation with soybean, were implemented in the last decade, changes in the agroecosystem of sugarcane, specific pathosystems and epidemiological parameters have been observed. A 16S metagenomics approach to investigate total bacterial communities associated with sugarcane rhizospheric soil when soybean was the predecessor crop in a cultivation area with a high incidence of red stripe disease was applied; soil from sugarcane monoculture was also included. Two commercial sugarcane cultivars (*Saccharum* spp. hybrids) with differential responses to red stripe infection (tolerant and susceptible) were evaluated. Sampling was carried out in 2013-2014 and in 2014-2015 (first and second ratoon, respectively) at 30, 90 and 180 days after harvest. Total soil DNA was obtained using FastPrep® technology. The 16S RNA gene (variable region V3-V4) was sequenced using a MiSeq platform Illumina. Taxonomic assignment revealed Bacillales, Rhyzobiales, Rhodospirilliales, Xanthomonadales and Acidobacteriales among the most abundant orders in all samples. Soil samples from sugarcane without soybean rotation showed a marked decrease in Bacillaes, Rhizobiales and Sphingomonadales. Cluster analysis grouped together samples from the tolerant genotype, while those from the susceptible genotype formed two subgroups that were distinguished according to sampling time after harvest. The analysis showed that samples from sugarcane under monoculture were grouped distant to the rest of the samples showing different microbiota composition. The sugarcane rhizosphere microbiome and its biotechnological potential open a new opportunity in the concept of sustainable crop management. The data contribute significant knowledge about the microbial diversity in agricultural ecosystems.

Key words Sugarcane, crop rotation, rhizospheric soil, microbial diversity, metagenomics

INTRODUCTION

Sugarcane production in Argentina occurs in three regions: Tucumán, Northern (Salta and Jujuy) and Littoral (Santa Fe and Misiones), extending over about 376,223 ha (Benedetti 2018). Tucumán produces the 68% of total national production (Pérez *et al.* 2007), with cultivation distributed in different agroecological regions, from the Highland region in the west to the Lower Plains and Chaco-Pampas in the east (Sanzano and Fadda 2009).

During the last decade, new agricultural techniques have been implemented that induced changes in the sugarcane agroecosystem, specifically in pathosystems and epidemiological parameters (Fontana *et al.* 2013). Changes in

management systems, such as green-cane harvesting and crop rotation with soybean, resulted in greater deposition of crop residues that generated changes in the soil conditions favoring the increase of many pathogens.

An increase of the red stripe disease incidence in commercial cultivars in the Northwest production areas has been seen in the last 15 years. In particular, the central-eastern zone in Tucumán (Burruyacú and Cruz Alta) was reported by Pérez Gómez *et al.* (2010) as the area with the highest incidence of red stripe. Fandos *et al.* (2013) reported that Burruyacú and Cruz Alta are the areas with the highest percentage of soybean rotation in the last 10 years. Soybean could generate changes in soil composition, resulting in a contribution of nutrients, especially nitrogen (N), which would increase the soil fertility, favoring not only crop growth but also allowing establishment of pathogenic bacteria in the sugarcane agroecosystem. Johnson *et al.* (2016) indicated that there is a positive correlation between the increase in the incidence of red stripe with several soil properties, including phosphorus, potassium and zinc levels.

Here, we applied a 16S metagenomics approach to investigate bacterial communities associated with sugarcane rhizospheric soil. We analyzed the cultivation system, with and without soybean as predecessor crop, in an area with a high incidence of red stripe disease.

MATERIALS AND METHODS

Experimental design

Sampling was carried out in the northeast of Tucumán Province (La Ramada, Burruyacu) in the Chaco-Pampeana SubHumid Plain Region (26°41´S, 64°56´W; 552 masl). It has gently undulating relief (soft hills), with soils of loam-silty texture, average annual rainfall of 750-1000 mm, evapotranspiration (ETP) of 900-950 mm, moderate to no water deficiency, average annual temperature of 19°C (January = 24°C; July = 12.5°C), and an average of 12 frosts per year (June to August).

Rhizosphere samples from each plot of both tolerant and susceptible sugarcane genotypes were collected in 2013-2014 and 2014-2015 (first and second ratoon, respectively) at 30, 90 and 180 days after harvest (Table 1). Before we collected the soil samples, the upper soil layer was scraped off to remove litter. After removing plant roots and large stones by sieving (1 mm sieve mesh), all soil samples were preserved in sterile tubes and stored at -20°C until use.

Bacterial community analysis

DNA extraction

DNA from 300 mg soil samples was extracted using the Fast DNA™ SPIN Kit for Soil (MP Biomedicals, USA) according to the manufacturer's protocol. Concentrations of double-stranded DNA in the extracts were determined using the Quant-iT dsDNA HS assay kit and the Qubit fluorometer (Invitrogen, Carlsbad, USA). The extracted nucleic acids were stored at -20°C for further analysis.

DNA amplification and Illumina high-throughput sequencing

PCR amplifications of the bacterial V3-V4 16S rRNA regions were carried out using the Phusion Flash High-Fidelity Master Mix (Thermo Fisher Scientific, Inc., Waltham, USA) and the 343F (5'-TACGGRAGGCAGCAG-3') and 802R (5'-TACNVGGGTWTCTAATCC-3') primer pairs (Polka *et al.* 2015).

A two-step PCR program with a first step in which untagged PCR primers amplify the template to desired yields was used. A dilution of the amplicons from the first step then served as a template in a successive low-cycle number using the barcoded primers.

PCR products were visualized by 1% (w/v) agarose gel electrophoresis then multiplexed into a single pool using equal molecular weights and purified with the Agencourt® AMPure® XP kit (Beckman Coulter, Italy). The sequencing was carried out in PTP-Science Park (Lodi, Italy) with a MiSeq Illumina instrument (Illumina Inc., San Diego, USA) generating 300 bp paired-end reads.

Table 1. Soil samples analyzed.

Sample ID	Condition	Date	Sampling day	Management
S64	GTIA	Oct 2013	30 dah	With rotation
S66	GTIA	Oct 2013	30 dah	With rotation
S65	GTIIA	Oct 2013	30 dah	With rotation
S67	GTIIA	Oct 2013	30 dah	With rotation
S62	GTIB	Dec 2013	90 dah	With rotation
S63	GTIIB	Dec 2013	90 dah	With rotation
S58	GTIC	Mar 2014	180 dah	With rotation
S59	GTIIC	Mar 2014	180 dah	With rotation
S74	GTID	Nov 2014	30 dah	With rotation
S77	GTIID	Nov 2014	30 dah	With rotation
S72	GTIE	Jan 2015	90 dah	With rotation
S75	GTIIE	Jan 2015	90 dah	With rotation
S73	GTIF	May 2015	180 dah	With rotation
S76	GTIIF	May 2015	180 dah	With rotation
S85	GTUG	May 2015	180 dah	Without rotation
S68	GSIA	Oct 2013	30 dah	With rotation
S70	GSIA	Oct 2013	30 dah	With rotation
S69	GSIIA	Oct 2013	30 dah	With rotation
S71	GSIIA	Oct 2013	30 dah	With rotation
S60	GSIB	Dec 2013	90 dah	With rotation
S61	GSIIB	Dec 2013	90 dah	With rotation
S56	GSIC	Mar 2014	180 dah	With rotation
S57	GSIIC	Mar 2014	180 dah	With rotation
S80	GSID	Nov 2014	30 dah	With rotation
S83	GSIID	Nov 2014	30 dah	With rotation
S78	GSIE	Jan 2015	90 dah	With rotation
S81	GSIIE	Jan 2015	90 dah	With rotation
S79	GSIF	May 2015	180 dah	With rotation
S82	GSIIF	May 2015	180 dah	With rotation
S84	GSUG	May 2015	180 dah	Without rotation

GT tolerant genotype; GS susceptible genotype; A, B, C, D, E, and F, treatments expressed in days after harvest (dah) during the 2013-2014 and 2014-2015 consecutive crops.
UG: Sugarcane without rotation (single sample)

Bioinformatics and statistical analysis

Raw reads were processed to trim adapters and low-quality sequences using Trimmomatic (version 0.33) using a sliding window of 30 bp with an average quality of Q28 and a minimum read length of 150 (Bolger *et al.* 2014). Reads were assembled and analyzed using the Mothur software package version 1.39.5 (Schloss *et al.* 2009). Sequences were classified using the references Ribosomal Database Project database (RDP) provided in Mothur. Rare OTUs (> 0.01%) were filtered out. Samples were normalized by rarefaction to the size of the smallest sample. Alpha diversity (InvSimpson, Chao and Shannon indices) was analyzed after normalization using Mothur. The R package DESeq2 (Love *et al.* 2014) was used to carry out comparative analysis to identify which OTUs changed significantly between different conditions.

Beta diversity analysis based on Bray Curtis similarity index was performed to investigate differences in the microbiota composition among samples. In addition, principal coordinate analysis (PCoA) was carried out to compare microbial composition among samples from different genotype conditions (GT and GS) and to assess both management systems (rotation and monoculture).

RESULTS

OTU-based analyses

After quality control, data were rarified to 12.155 sequences per sample (20 after adjustment) and assured a > 99% coverage for all samples. Taxonomical assignment showed that the 99.4% of sequences were accurately classified at genus level and 94.4% at species level. OTU data were filtered with a cut-off of 99.9%. DESeq analysis showed that 202 OTUs (based on 1200 total OTUs identified) were differentially represented between tolerant and

susceptible genotypes, with adjusted p-values ≤ 0.01 and a fold change ≥ 1 which were considered significantly differentially abundant.

α-diversity indexes

Achieved coverage of the identified total bacterial diversity according to the dedicated sequencing effort in the data analysis was over 95%. α-diversity analysis considering genotype condition (Table 2) showed that samples were very similar based on OTU-richness at different sampling time. When monoculture and rotation system were compared (only samples from 180 days after harvest), differences were more evident. Richness and abundances index showed that rotation systems were more diverse than monoculture system (Table 2).

Table 2. Summary of alpha diversity results for all samples analyzed.

Sample	Condition	nseqs	OTUs	InvSimpson	Chao	Shannon
With rotation						
S66	GTIA	12155	1038	168.80	1119.39	6.03
S67	GTIIA	12155	1017	115.56	1094.83	5.89
S72	GTIE	12155	1027	110.39	1126.51	5.80
S73	GTIF	12155	962	72.71	1099.26	5.53
S74	GTID	12155	1017	91.99	1108.84	5.74
S75	GTIIE	12155	1018	99.87	1094.67	5.75
S76	GTIIF	12155	1025	106.02	1137.05	5.84
S77	GTIID	12155	977	97.07	1100.38	5.66
S68	GSIA	12155	1052	151.00	1123.02	6.01
S69	GSIIA	12155	1031	42.47	1100.71	5.59
S70	GSIA	12155	1065	131.96	1141.52	5.99
S71	GSIIA	12155	1036	43.18	1129.80	5.64
S78	GSIE	12155	1063	131.95	1148.99	5.95
S79	GSIF	12155	1028	89.99	1090.92	5.69
S80	GSID	12155	1050	158.48	1142.66	6.00
S81	GSIIE	12155	1056	158.26	1137.69	5.99
S82	GSIIF	12155	1020	147.55	1094.65	5.96
S83	GSIID	12155	1043	128.33	1094.44	5.91
Without rotation						
S84	GSUG	12155	721	99.38	859.49	5.44
S85	GTUG	12155	720	120.62	883.28	5.53

When relative abundance was calculated at phylum level (Figure 1), a decrease in the Fimicutes and Gemmatimonadetes and an increase in Chloroflexi in the samples of the system without rotation was seen. Within the phylum Fimicutes, a significant decrease in *Bacillus* genera, known for their role in production of antibiotics and biological control, was seen. Taxonomy-based analysis at order level showed high presence of Bacillales, Rhizobiales, Rhodospirillales, Sphingomonadales as predominant orders in all analyzed samples.

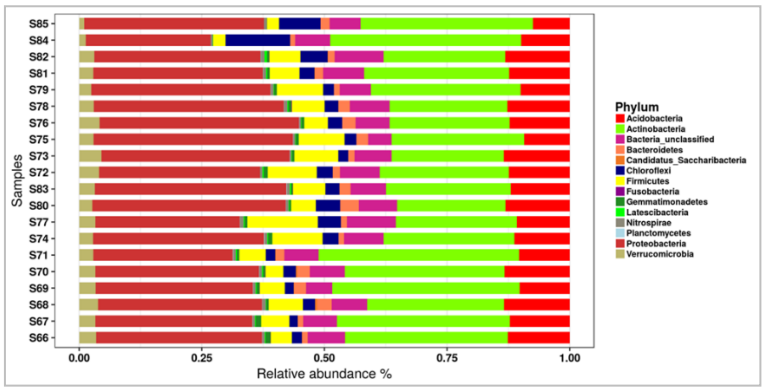

Figure 1. Relative abundance at Phylum level in the 20 samples analyzed.

β-diversity analysis

Beta diversity analysis based on the Bray Curtis similarity index was performed to investigate differences in the composition of the microbiota among samples from different conditions. Figure 2 shows the cluster analysis considering soil samples from tolerant and susceptible genotypes (GT and GS, respectively). GT samples were grouped in a single cluster, while GS were distributed in two clusters. Samples from October 2013 (30 days after harvest) were grouped at the top of graph, while in the second group 90-day and 180-day samples were mixed. S84 (GS) and S85 (GT) samples from the monoculture system were always in a single cluster and separated from the remainder.

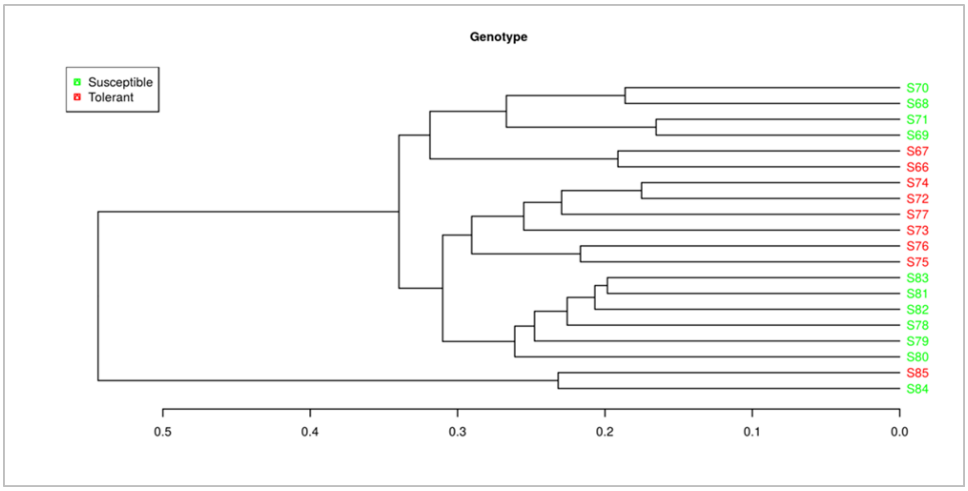

Figure 2. Cluster analysis of the samples according to genotype condition (tolerant and susceptible) using the Bray Curtis index and the UPGMA algorithm.

The principal coordinate analysis plot (PCoA) considering genotype (Figure 3) only from rotation system samples, indicated the existence of a dissimilarity in microbial composition among samples. Figure 4 shows the PCoA analysis for both rotation and monoculture system, which reveals the existence of a large dissimilarity in microbial composition among samples. All samples from rotation system tend to cluster together, as do those from monoculture.

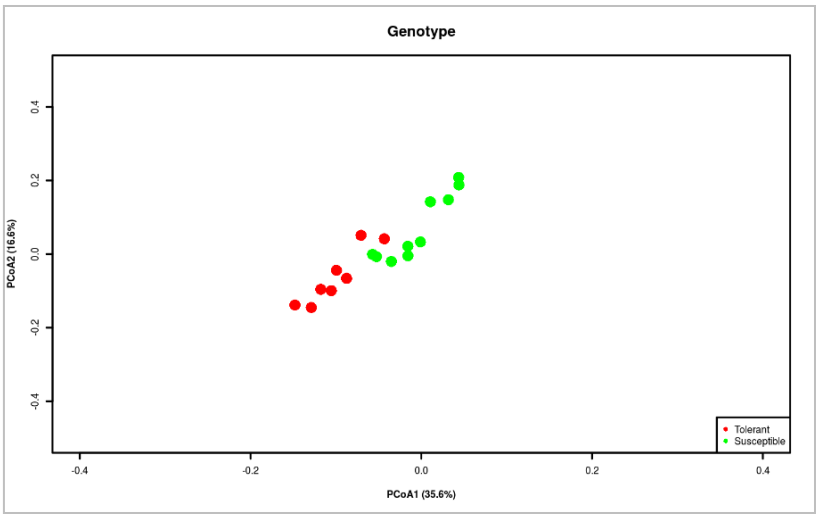

Figure 3. Principal coordinate analysis according to the genotype condition (tolerant and susceptible) in a rotation system.

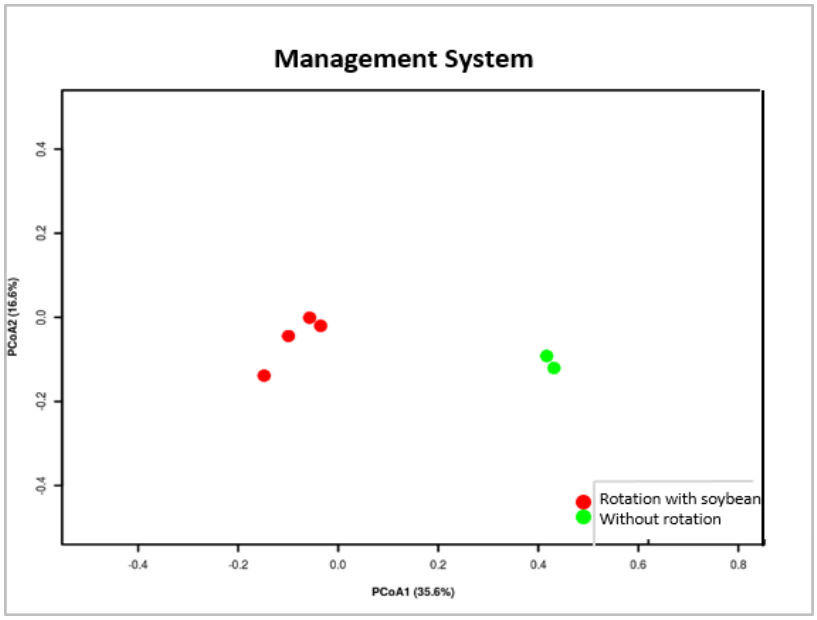

Figure 4. Principal coordinate analysis according to management strategy, rotation versus without rotation.

DISCUSSION

Currently, sugarcane agronomic and cultural management comprises green-harvesting (without previous burning of sugarcane) and soybean (*Glycine max* L.) crop rotation between successive sugarcane plantings. These practices may affect the behavior of a given pathosystem and/or epidemiological parameters. Sugarcane crop rotation with soybean has been common practice for more than 10 years (Fandos *et al.* 2013) in the central-eastern zone of Tucumán (Burruyacú and Cruz Alta). An increase in the incidence of sugarcane red stripe, caused by *Acidovorax avenae* (Fontana *et al.* 2013), has been observed in commercial plots in these areas (Perez Gomez *et al.* 2010).

To explore microbial communities, soil samples from the sugarcane agroecosystem were subjected to 16S metagenomics analysis. Our results describe the microbial composition of a sugarcane rhizospheric soil from Argentina based on high performance sequencing technology (HTS). The richness and abundance indicators for each sample analyzed were adequate to describe the biodiversity present in the system under study.

Metagenome approaches to agro-ecosystem analysis are relatively recent and can provide a large amount of data to understand processes that are still unknown and eventually answer some questions about the functionality and the role of microbial communities in the studied systems.

Souza *et al.* (2016) carried out the first work with a "microbiome" approach in different organs of sugarcane, namely roots, stems and leaves of plants grown in greenhouse and a single clone. They reported that more than 90% of OTUs in roots, stems and leaves were also in bulk soil samples, suggesting that microorganisms in bulk soil colonize plant organs at early stages of plant development and the organ-specific richness and abundance may be dictated by functional or adaptive plant-microbe interaction (Souza *et al.* 2016).

In our study, the alpha diversity index showed no significant differences among samples based on genotypes, cultivation systems or sampling period. Taxonomic assessment showed a slight increase in Sphingomonadales, Bacillales and Rhizobiales abundance in the tolerant genotype (GT) at 90-day and 180-day samplings in both crop cycles, while a marked decrease in Bacillaes, Rhizobiales, Sphingomonadales and Chthoniobacteriales in the monoculture system at 180 days after harvest for both genotypes was observed. Similar to our findings, Souza *et al.* (2016) reported that genera such as *Azospirillum, Bacillus, Bradyrhizobium, Enterobacter*, described as plant growth promoters (PGPB) in many plants, are presented as secondary components of the sugarcane microbiome.

Although crop rotation benefits have been widely studied due to the improvements in soil structure, physics and chemistry, microbiological aspects have not frequently been addressed. Data generated in our study constitutes an important advance in the knowledge of the "not always visible" benefits of crop rotation in agricultural systems

and particularly in sugarcane. Differences in the microbial composition in the tolerant and susceptible to red stripe genotypes were observed. Nevertheless, more studies are needed, oriented to the possible mechanisms involved in plant response.

Relationships between sugarcane microbiota and factors such as variety, crop cycle or a particular disease has also been studied by other groups of researchers from important sugarcane regions of the world. Recently, Hamonts *et al.* (2018) showed that multiple factors interact in complex ways in the communities of each part of the plant. Different bacteria and fungi associations were determined mainly by the part of the plant analyzed, followed by the growing region, the age of the crop, the variety of sugarcane and by the yellowing of the leaves syndrome or Yellow Canopy Syndrome (YCS). Bacterial communities of rhizosphere roots and soil are more influenced by the cultivation region, whereas bacterial communities of the stem and leaves are mostly affected by the cultivar and age of the crop.

Approaches based on the microbiome from economically important crops are being developed at present. In particular, for sugarcane, the potential benefit of microorganisms present in the microbiome constitutes a great challenge, based on the importance and need to increase productivity in a sustainable agro-ecosystem (Souza *et al.* 2016; Yeoh *et al.* 2016). On the other hand, the understanding of relationships between microbiome and a particular disease would provide important data that would allow in the long term the identification of possible management strategies for a specific pathogen, including an adjusted breeding program, or a better prediction of management results (Hamonts *et al.* 2018). Microbial communities associated with plants can also protect, improve growth through the production of phytohormones and help plants with different stresses (Ali *et al.* 2009, Innerebner *et al.* 2011, Dong *et al.* 2018).

The nitrogen-fixing capacity of the microbiome associated with sugarcane may become an alternative to the application of nitrogen fertilizers and has been extensively studied, especially for endophytic diazotrophs (Elbeltagy *et al.* 2001; Souza *et al.* 2016). Recent studies showed that the application of nitrogenous fertilizer did not change the central microbial community in sugarcane varieties and that plant genotype only had a subtle effect on the composition of the community (Yeoh *et al*. 2016).

The origin of the microbiome, and the dynamics and the assembly patterns are important for the elucidation of its possible role in growth, development and response of plants to biotic and abiotic stresses.

CONCLUSIONS

Based on our results, we can infer that there are very important differences in the composition and structure of the bacterial communities in the analyzed samples. Rotation with soybean implies a richness and abundance of communities that can improve the nutrition of the plant and therefore favors the development of diseases. Our study revealed that crop management crops strategies had effects on microbial community structures.

REFERENCES

Ali B, Sabri AN, Ljung K, Hasnain S. 2009. Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum* L. *Letters in Applied Microbiology* 48: 542–547.

Benedetti P. 2018. *Primer relevamiento del cultivo de caña de azúcar de la República Argentina a partir de imágenes satelitales para la campaña 2018*. http:/latamsatelital.com/primer-relevamiento-del-area-cultivada-cana-argentina-traves-imagenes-satelitales/ (accessed 7 September 2018).

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014; btu170.

Dong M, Yang Z, Cheng G, Peng L, Xu Q, Xu J. 2018. Diversity of the bacterial microbiome in the roots of four *Saccharum* species: S*. spontaneum*, *S. robustum*, *S. barberi*, and *S. officinarum*. *Frontiers in Microbiology* 9: 267.

Elbeltagy A, Nishioka K, Sato T, Suzuki H, Ye B, Hamada T. 2001. Endophytic colonization and in planta nitrogen fixation by a *Herbaspirillum* sp. isolated from wild rice species. *Applied and Environmental Microbiology* 67: 5285–5293.

Fandos C, Soria FJ, Scandaliaris P, Carreras Baldrés, JI. 2013. Análisis departamental del proceso de expansión del cultivo de caña de azúcar en el área granera tucumana. Período febrero 2001-mayo 2013. In *El cultivo de la soja en el Noroeste Argentino. Campaña 2012/2013* (Devani R, Ledesma F, Sánchez JR Eds), pp. 203–208. Publ. Espec. EEAOC 47. EEAOC, Tucumán.

Fontana PD, Rago AM, Fontana CA, Vignolo GM, Cocconcelli PS, Mariotti JA. 2013. Isolation and genetic characterization of *Acidovorax avenae* from red stripe infected sugarcane in northwestern Argentina. *European Journal of Plant Pathology* 137: 525-534.

Hamonts K, Trivedi P, Garg A, *et al*. 2018. Field study reveals core plant microbiota and relative importance of their drivers. *Environmental Microbiology* 20: 124–140.

Innerebner G, Knief C, Vorholt JA. 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Applied and Environmental Microbiology* 77: 3202–3210.
Johnson RM, Grisham MP, Warnke KZ, Maggio JR. 2016. Relationship of soil properties and sugarcane yields to red stripe in Louisiana. *Phytopathology* 106: 737–744.
Love ML, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with deseq2. *Genome Biology* 15: 550.
Pérez D, Fandos C, Scandaliaris J, Mazzone L, Soria F, Scandaliaris P. 2007. *Estado actual y evolución de la productividad del cultivo de caña de azúcar en Tucumán y el noroeste argentino en el período 1990-2007*. Informe Especial EEAOC 34. EEAOC, Tucumán.
Pérez Gómez S, Vallejo J, Fontana P, Rago A. 2010. Evaluación de estría roja en los cañaverales de Tucumán. In *XVI Reunión Técnica Nacional de la Caña de Azúcar, Tucumán, Argentina*. Resumen Nº 48.
Połka J, Rebecchi A, Pisacane V, Morelli L, Puglisi E. 2015. Bacterial diversity in typical Italian salami at different ripening stages as revealed by high throughput sequencing of 16S rRNA amplicons. *Food Microbiology* 46: 342–356.
Sanzano A, Fadda G. 2009. Características de los suelos para caña de azúcar. Recomendaciones de manejo. In *Manual del cañero* (Romero E, Dogonzelli P, Scandaliaris J Eds), pp. 23–34. EEAOC, Tucumán.
Schloss P, Westcott S, Ryabin T, *et al*. 2009. Introducing Mothur: open-source, platform independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75: 7537–7541.
Souza RSC, Okura VK, Armanhi JSL, *et al*. 2016. Unlocking the bacterial and fungal community assemblages of sugarcane microbiome. *Scientific Reports* 6: 28774.
Yeoh YK, Paungfoo-Lonhienne C, Dennis PG, *et al*. 2016 The core root microbiome of sugarcanes cultivated under varying nitrogen fertilizer application. *Environmental Microbiology* 18: 1338–1351.

Réponses de la communauté microbienne du sol à différentes stratégies de gestion de la canne à sucre révélées par la métagénomique 16S

Résumé. La culture de la canne à sucre en Argentine est répartie dans trois régions géographiques: Tucumán, le Nord (Salta et Jujuy) et le Littoral (Santa Fe et Misiones), couvrant environ 376 223 ha. Tucumán est traditionnellement la région la plus importante avec 68% de la production totale. Depuis que de nouvelles techniques agricoles, telles que la récolte de la canne verte et la rotation de la canne à sucre avec le soja, ont été mises en place au cours de la dernière décennie, des changements dans l'écosystème de la canne à sucre, des pathosystèmes spécifiques et des paramètres épidémiologiques ont été observés. Une approche métagénomique 16S pour étudier les communautés bactériennes totales associées au sol rhizosphérique de la canne à sucre lorsque le soja était la culture précédente dans une zone de culture présentant une incidence élevée de maladie à rayures rouges a été appliquée; le sol issu de la monoculture de canne à sucre a également été inclus. Deux cultivars commerciaux de la canne à sucre (hybrides *Saccharum* spp.) avec des réponses différentielles à l'infection à rayures rouges (tolérants et sensibles) ont été évalués. L'échantillonnage a été réalisé en 2013-2014 et en 2014-2015 (première et deuxième repousses, respectivement) à 30, 90 et 180 jours après la récolte. L'ADN total du sol a été obtenu en utilisant la technologie FastPrep®. Le gène de l'ARN 16S (région variable V3-V4) a été séquence en utilisant une plate-forme MiSeq Illumina. L'analyse taxonomique a révélé que Bacillales, Rhyzobiales, Rhodospirilliales, Xanthomonadales et Acidobacteriales étaient parmi les ordres les plus abondants dans tous les échantillons. Des échantillons de sol de canne à sucre sans rotation de soja ont montré une diminution marquée des bacilles, des rhizobiales et des sphingomonadales. L'analyse a regroupé des échantillons du génotype tolérant, tandis que ceux du génotype sensible ont formé deux sous-groupes qui ont été distingués en fonction de la période d'échantillonnage après la récolte. L'analyse a montré que les échantillons provenant de la canne à sucre en monoculture étaient regroupés à distance du reste des échantillons présentant une composition de microbiote différente. Le microbiome de la rhizosphère de canne à sucre et son potentiel biotechnologique ouvrent une nouvelle opportunité dans le concept de gestion durable des cultures. Les données fournissent des connaissances significatives sur la diversité microbienne dans les écosystèmes agricoles.

Mots-clés: Canne à sucre, rotation des cultures, sol rhizosphérique, diversité microbienne, métagénomique

Respuestas de la comunidad microbiana del suelo a diferentes estrategias de manejo de la caña de azúcar según lo revelado por 16S metagenómica

Resumen. El cultivo de caña de azúcar en Argentina se distribuye en tres regiones geográficas: Tucumán, Norte (Salta y Jujuy) y Litoral (Santa Fe y Misiones), que cubren alrededor de 376,223 ha. Tucumán ha sido tradicionalmente la región más importante con el 68% de la producción total. En la última década, como consecuencia de los cambios en el manejo como la rotación con soja y la cosecha en verde, se han observado cambios en el agroecosistema de la caña de azúcar, en patosistemas específicos y parámetros epidemiológicos. En el presente estudio, se aplicó un enfoque metagenómica de gen 16S ARNr para investigar las comunidades bacterianas asociadas con el suelo rizosférico de caña de azúcar en un sistema con soja como cultivo predecesor en un área de cultivo con una alta incidencia de estría roja. A fines comparativos, se incluyó en el estudio muestras de suelo monocultivo de caña de azúcar. Se evaluaron dos cultivares comerciales de caña de azúcar (*Saccharum* spp. híbridos) con respuestas diferenciales a la infección de estría roja (tolerante y susceptible). A partir del ADN total del suelo de muestras obtenidas en las campañas 2013-2014 y en 2014-2015 (soca 1 y 2, respectivamente) a los 30, 90 y 180 días después de la

cosecha, se amplificó la región variable V3-V4 del 16S rRNA y se secuenció utilizando una plataforma MiSeq Illumina. La asignación taxonómica de las secuencias, reveló Bacillales, Rhyzobiales, Rhodospirilliales, Xanthomonadales y Acidobacteriales entre las órdenes más abundantes en todas las muestras. Las muestras de suelo de caña de azúcar sin rotación con soja mostraron una marcada disminución en Bacillaes, Rhizobiales y Sphingomonadales. El análisis de conglomerados agrupó las muestras del genotipo tolerante, mientras que las del genotipo susceptible formaron dos subgrupos que se distinguieron según el tiempo de muestreo después de la cosecha. El análisis mostró además que las muestras de caña de azúcar provenientes de un sistema de monocultivo se agruparon distantes del resto de las muestras evidenciando las diferencias en la composición de microbiota. El microbioma de la rizosfera de la caña de azúcar y su potencial biotecnológico abren una nueva oportunidad en el concepto de manejo sostenible de cultivos. Los datos aportan un conocimiento significativo sobre la diversidad microbiana en los ecosistemas agrícolas.

Palabras clave: Caña de azúcar, rotación de cultivos, suelo rizosférico, diversidad microbiana, metagenómica