

## Changes in the bacterial community structure in soil under conventional and conservation practices throughout a complete maize (*Zea mays* L.) crop cycle

Edson A. Romero-Salas<sup>a</sup>, Yendi E. Navarro-Noya<sup>b</sup>, Marco Luna-Guido<sup>a</sup>, Nele Verhulst<sup>c</sup>, José Crossa<sup>c</sup>, Bram Govaerts<sup>c,d</sup>, Luc Dendooven<sup>a,\*</sup>

<sup>a</sup> Laboratory of Soil Ecology, ABACUS, Cinvestav, Mexico City 07360, Mexico

<sup>b</sup> CONACYT-Tlaxcala Autonomous University, Tlaxcala, Tlaxcala 90000, Mexico

<sup>c</sup> International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico City, Mexico

<sup>d</sup> Cornell University, Ithaca, USA

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### ABSTRACT

Agricultural practices and changes in soil conditions, such as water content, inorganic N content, temperature, pH and organic material availability, affect the bacterial community structure. Soil characteristics and the bacterial community structure were monitored in soil with maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) rotation, zero tillage and crop residue kept (ZTRK) or removed (ZTRR), and conventional tillage with monoculture maize (CTMR) or maize-wheat rotation and crop residue removed (CTRR) or kept in the field and ploughed in (CTRK). The soil organic C was significantly affected by tillage practices and decreased ZTRK > CTRK > CTRR = ZTRR > CTMR, while water content and NO<sub>3</sub><sup>-</sup> concentration showed large fluctuations over the crop cycle, but were not affected significantly by agricultural practices. The bacterial community structure showed large changes over the crop cycle determined by varying soil characteristics, most importantly water content and NO<sub>3</sub><sup>-</sup> concentration and six bacterial genera, i.e. *Achromobacter*, *Bacillus*, *Halomonas*, *Kaistobacter*, *Pseudomonas* and *Serratia*, while changes due to agricultural practices were much smaller. It was found that the bacterial community structure was affected significantly by time, tillage (zero tillage versus conventional tillage), crop residue management (kept versus removed) and crop rotation (CTMR versus CTRR treatment).

### 1. Introduction

Soil conditions, such as pH, water content, temperature, available organic material and N fertilizer application, control the bacterial community structure (Jangid et al., 2008; Fierer, 2017). Complex interactions between these factors drive changes in soil bacterial communities and their functioning (Wu et al., 2015; Xue et al., 2018). Water content is one of the major factors affecting soil microorganisms (Wang et al., 2019). An excess of water reduces gas fluxes and results in a decrease in oxygen (O<sub>2</sub>) content in soil, creating anaerobic conditions, which favors facultative and obligate anaerobes; while a lack of water enriches microorganisms that can survive with little or no water (Yan et al., 2015; Schimel, 2018). Microbial activity is affected by temperature, but the optimum for growth is highly variable between them. Organic material left in the field, such as crop residue, facilitates water infiltration, prevents erosion and limits evaporation, but also serves as a carbon source for heterotrophs (Fernandez et al., 2016). The

composition and availability of the organic material will determine which microorganisms will be enriched in soil (Valboa et al., 2015; Zhang et al., 2016). Copiotrophs are enriched when easily decomposable organic material is applied to soil, while oligotrophs are enriched in nutrient poor environments (Giovannoni et al., 2014). Crop residue removal or leaving it on the soil surface limits the amount of C substrate available for heterotrophs, while ploughing brings it in direct contact with microorganisms accelerating organic material mineralization (Carbonetto et al., 2014; Dimassi et al., 2014). Tillage breaks up aggregates so the organic material physically protected becomes available for microorganisms thereby reducing the soil organic matter content (Abdollahi and Munkholm, 2014; Shahbaz et al., 2017). Inorganic N fertilizer application, such as urea or ammonium, might stimulate the activity of nitrifiers (Wu et al., 2011). Additionally, some soil organic material is low in N, i.e. it has a high C-to-N ratio, and so its mineralization is delayed (Marschner et al., 2003; Wang et al., 2014). Application of inorganic N fertilizer will increase the soil mineral N

\* Corresponding author.

E-mail address: [dendooven@me.com](mailto:dendooven@me.com) (L. Dendooven).

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**Abbreviations**

16S rRNA 16S ribosomal ribonucleic acid

CA conservation agriculture

CIMMYT International Maize and Wheat Improvement Centre

CP conventional practices

DNA deoxyribonucleic acid

EC electrolytic conductivity

OTU Operational taxonomic units

PCR polymerase chain reaction

TOC total organic C content

WC soil moisture content

WHC water holding capacity

content, which might accelerate the degradation of organic material with a high C-to-N ratio.

The “International Maize and Wheat Improvement Centre (CIMMYT)” manages long-term field experiments that study the effect of conservation agriculture (CA) on crop yields and soil characteristics, with the two longest running experiments initiated in the early 1990s (Govaerts et al., 2008; Verhulst et al., 2011a). Conservation agriculture combines crop rotation with zero tillage and maximum retention of crop residue in the field. This contrasts with conventional practices (CP) that use tillage, incorporation or removal of crop residue and in Mexico, monoculture of maize (*Zea mays* L.). These contrasting agricultural practices affect yields, but also soil characteristics and the microbial community structure (Navarro-Noya et al., 2013; Pittelkow et al., 2015). In a previous study in which the effect of agricultural practices, crop residue management, crop rotation and tillage, on the bacterial community was studied; the relative abundance of Actinobacteria, Betaproteobacteria and Gammaproteobacteria was affected significantly by tillage and correlated to the total organic carbon and clay content in soil (Navarro-Noya et al., 2013). Removal or retaining crop residue management in the field had also a significant effect on the bacterial community structure, but not crop rotation (maize – wheat) or monoculture of maize. It remains to be seen if the effect of crop residue management on the bacterial community structure prevails during an entire crop cycle when soil conditions change.

Soil temperature, pH, water content and available organic material

changes in an agricultural cycle, i.e. the annual cycle of activities related to the growth and harvest of a crop, due to the climate and practices applied and this will alter the bacterial community, but to what extent is still largely unknown (Pasternak et al., 2013; Orellana et al., 2018). Therefore, soil was sampled extensively (i.e. 18 times over a year (Fig. 1)) from five treatments at El Batán in the central highlands of Mexico during a complete crop cycle and the preceding dry fallow period, while soil characteristics and the bacterial community structure were monitored. The five treatments monitored were soil with maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) rotation, zero tillage and crop residue kept (ZTRK) or removed (ZTRR), and conventional tillage with monoculture maize (CTMR) or maize-wheat rotation and crop residue removed (CTRR) or kept in the field and ploughed in (CTRK) (Table S1). It was hypothesized that the bacterial community would change over a crop cycle, but the effect of agricultural practices on it would be preserved.

**2. Materials and methods****2.1. Experimental site**

The experimental site is located at El Batán (Texcoco, State of Mexico) in the subtropical highlands of Central Mexico (2240 masl, 19.31°N, 98.50°W) (Govaerts et al., 2008). The mean maximum and minimum temperatures at the experimental site are 24 °C and 7 °C,

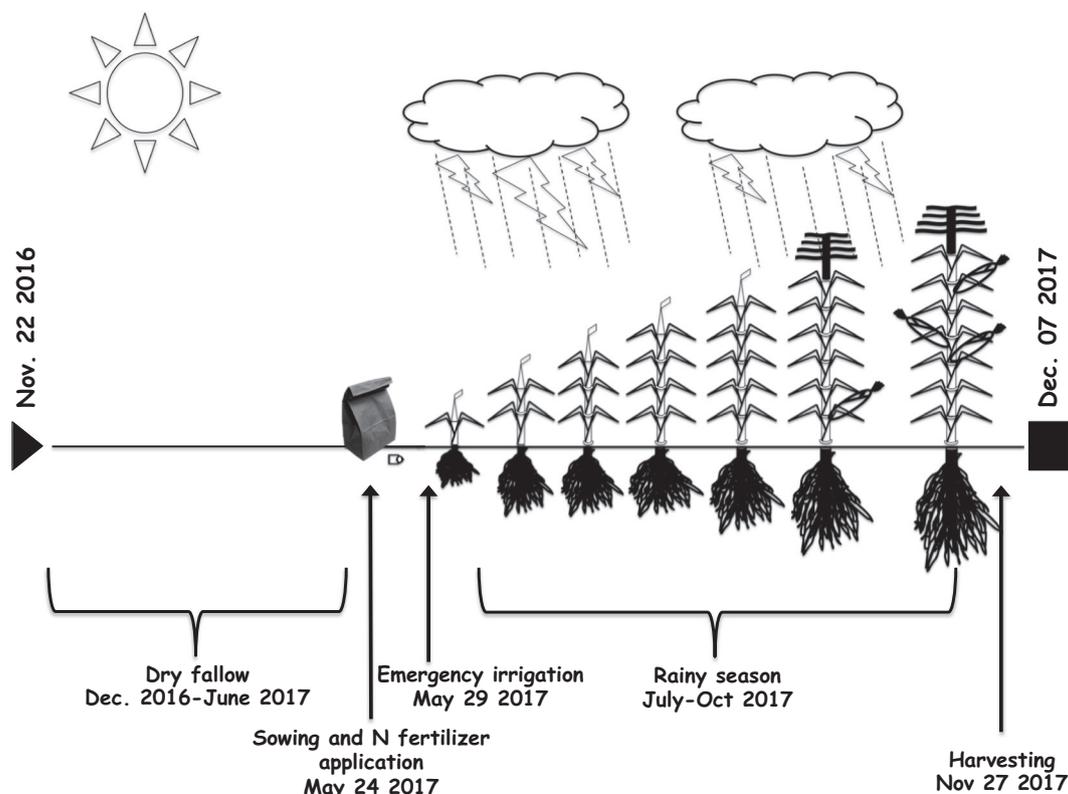


Fig. 1. A crop cycle at the field experiment in El Batán (Mexico).

respectively (1991–2018) and the average annual rainfall is 651 mm, with on average 558 mm falling between May and October (Figs. S1, S2). Short, intense rain showers followed by dry spells typify the summer rainy season and the total yearly potential evapotranspiration of 1538 mm exceeds annual rainfall. The El Batán experimental station has an average growing period of 132 days. The soil at the experimental site is classified as a Haplic Phaeozem (Calyic) in the world reference base system (IUSS Working Group WRB, 2006) and as a fine, mixed, thermic Cumulic Haplustoll in the USDA Soil Taxonomy system (Soil Survey Staff, 2003).

## 2.2. Treatments at the experimental site

The rainfed long-term experiment began in 1991, to study the effects of different tillage, sowing and residues management, as well as maize and wheat crop rotation (alternating years) on crop yields (Govaerts et al., 2008). The experimental design was a randomized complete block design with two field replicates and 32 treatments. In this study, five treatments were sampled including conventional agricultural practices (conventional tillage, monoculture maize crop (considered the CTMR treatment) or wheat-maize rotation, crop residues kept (considered the CTRK treatment) or removed (considered the CTRR treatment) and conservation agricultural practices (zero tillage, maize-wheat crop rotation and crop residues kept (considered the ZTRK treatment) or removed (considered the ZTRR treatment)) (Table S1). All treatments sampled in this study were cultivated with maize and mean yields ( $\text{kg ha}^{-1}$  at 12% $\text{H}_2\text{O}$ ) for the years 1997–2002 were: CTMR  $3570 \pm 220$ , CTRR  $4063 \pm 388$ , CTRK  $4403 \pm 150$ , ZTRR  $4339 \pm 461$ , ZTRK  $5285 \pm 184$  (Govaerts et al., 2005). Details on tillage and residue management can be found in Verhulst et al. (2011b).

Urea was surface-banded at sowing at a rate of  $150 \text{ kg N ha}^{-1}$ , as well as  $40 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  as triple superphosphate. In treatments with conventional tillage, weeds were controlled by tillage during the dry season (two to three times in total), whereas in zero tillage treatments glyphosate was used with approximately the same frequency. The use of selective herbicides was the same in all treatments. Disease or insect pest controls were only used when necessary in all treatments to save the experiment and as seed treatments applied by commercial seed sources. Maize was planted at  $75,000 \text{ plants ha}^{-1}$  and wheat at  $110 \text{ kg seed ha}^{-1}$  between mid-May and mid-June at the onset of summer rains. In 2017, maize was sown on May 24 (Fig. 1).

## 2.3. Soil sampling and soil characterization

The experimental lay-out contained two replicated plots as the experiment was designed originally to study the effect of agricultural practices on crop yields on a long term base. The number of replicates used in a field experiment depends largely on the size and cost of the experiment. The practical number of replicates for an experiment is reached when the cost of the experiment is no longer offset by an increase in information gained (Steel and Torrie, 1980). Experimental designs in agronomy, plant breeding and agriculture, therefore, often use two replicates to reduce costs while still allowing to test for significant differences between treatments (Federer, 1977; Federer and Crossa, 2012). Two replicated plots have been used at El Batán since 1991 to reduce cost while providing enough information to determine which agricultural practices, i.e. conventional practices versus conservation agriculture, improve yields and how they affect soil characteristics, greenhouse gas emissions and bacterial populations (e.g. Navarro-Noya et al., 2013). Although the variation is larger when two replicates are used in a statistical analysis and “renders any test less powerful” (Fay and Gerow, 2013), it is perfectly possible to determine the effect of an agricultural treatment on yield, soil characteristics or microbial populations, as in this study (e.g. Basnet et al., 2019; Crespo-Herrera et al., 2018).

The 0–20 cm layer of two plots (size  $7.5 \times 22 \text{ m}$ ) per treatment was

sampled in eight points per plot (approximately 500 g) between the rows of maize plants (distance between the rows was 0.75 m) with a  $\varnothing$  2 cm soil auger (Fig. S3). The soil from each plot was pooled (O'Brien et al., 2016) so that 10 soil samples ( $n = 10$ ) were obtained on each sampling day, i.e. 2 plots ( $n = 2$ ) from 5 treatments ( $n = 5$ ). Overall, soil was collected 18 times over the crop cycle (Fig. 1). The soil was sampled seven times (once per month) during the dry fallow part of the crop cycle, i.e. from November 2016 to May 2017. The sampling intensity was increased to twice per month during the rainy season, i.e. from June to October 2017, which corresponds with crop growth, while a final post harvest sampling took place in December 2017. It was assumed that taken 18 samples over a crop cycle was sufficient to determine changes in the bacterial community structure. The organic material on the soil surface of the treatments where the crop residue was kept (CTRK and ZTRK), was removed before the soil samples were taken. Soil samples were collected, placed in sterile dark plastic bags and kept on ice  $< 4 \text{ h}$ , until analyzed. Each soil sample was sieved to 2 mm and approximately 50 g were stored at  $-80 \text{ }^\circ\text{C}$  for DNA extraction.

At the onset of the experiment the soil samples were analyzed for particle size distribution with the Bouyoucus method (Gee and Bauder, 1986) and water holding capacity (WHC) (Table S1). The WHC was defined as the water retained by a dry soil left to drain freely overnight (Cassel and Nielsen, 1986). Each soil sample taken between 22 November 2016 and 7 December 2017 was analyzed for organic carbon content as described by Amato (1983), electrolytic conductivity (EC) by the saturated soil-paste method (Rhoades et al., 1989) and pH in a soil suspension (Thomas, 1996). The mineral N content ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ ) was determined according to Mulvaney (1996).

## 2.4. DNA extraction and PCR amplification of bacterial 16S rRNA gene

Each soil sample was washed with 0.15 M sodium pyrophosphate and 0.15 M phosphate buffer pH 8 to remove fulvic and humic acids (Ceja-Navarro et al., 2010). Three different techniques were used to extract the DNA from the washed soil and the DNA obtained with each method was pooled per sample. Each technique was used to extract DNA from a 2 g sub-sample of soil. As such, DNA was extracted from 6 g soil per plot and 12 g per treatment. The first method used was based on the technique described by Ceja-Navarro et al. (2010) and consisted of a chemical and thermal shock for cell lysis. The second method was developed by Sambrook and Russell (2001) and cells were enzymatically lysed, while the third method used a detergent solution and mechanic disruption for cell lysis as described by Hoffman and Winston (1987).

The V3-V4 region of the 16S rRNA bacterial gene was amplified with 8-bp barcoded primers 341-F (5'CCTACGGGNGGCWGCAG 3') and 805-R (5'GACTACHVGGGTATCTAATCC 3') (Herlemann et al., 2011). The PCRs were done as previously described by Navarro-Noya et al. (2013). The product of three reactions of each metagenomic DNA sample was pooled to minimize PCR bias and made a single library (Acinas et al., 2004). All libraries were purified using the DNA Clean and Concentrator purification kit as recommended by the manufacturer (Zymo Research, Irvine, CA, USA), and quantified using the PicoGreen® dsDNA assay (Invitrogen, Carlsbad, CA, USA) and the NanoDrop 3300 Fluorospectrometer (Thermo Scientific NanoDrop; Thermo Fisher Scientific, Wilmington, DE, USA). The libraries were normalized and pooled, and subsequently sequenced with 300-bp paired-end (PE) runs MiSeq runs (Illumina) by Macrogen, Inc. (DNA Sequencing Service, Seoul, Korea).

## 2.5. Analysis of Illumina sequencing data

The bacterial 16S rRNA gene sequences were analyzed with QIIME version 1.9.1 software (Caporaso et al., 2010b). Paired-end sequences were assembled with fastq-join method within QIIME. Trimmed reads were demultiplexed, and chimeras and those with a low quality were

removed (i.e. less than 19 Phred score). Operational taxonomic units (OTU) with 97% similarity were determined using the Greengenes v13.8 database with the UCLUST algorithm (Edgar, 2010). Sequences were aligned against the Greengenes core set using representative sequences of each OTU with PyNAST and filtered at a 75% threshold (Caporaso et al., 2010a). One representative sequence of each OTU was selected and the taxonomic assignment determined using the naïve Bayesian classifier tool from the ribosomal database project release 11 (<http://edp.cme.msu.edu/classifier/classifier.jps>) (Wang et al., 2007). Biological observations matrices (biom) with the taxonomic assignment and metadata of the soil samples were constructed and used in the next analysis. Samples with < 3000 sequences were discarded from the OTU-biom, and a total of 153 samples were used for further analysis. The OTU-biom was rarefied to the same sequence depth and was used in the alpha diversity analysis, i.e.  $n = 8000$  (Gilbert et al., 2009; O'Brien et al., 2016). The rarefied tables were obtained by consecutively sub-sampling the population of bacterial occurrences for each soil sample at intervals of 100 sequences between 100 and 3171 reads. This sequential rarefaction was done 50 times.

## 2.6. Diversity and statistical analysis

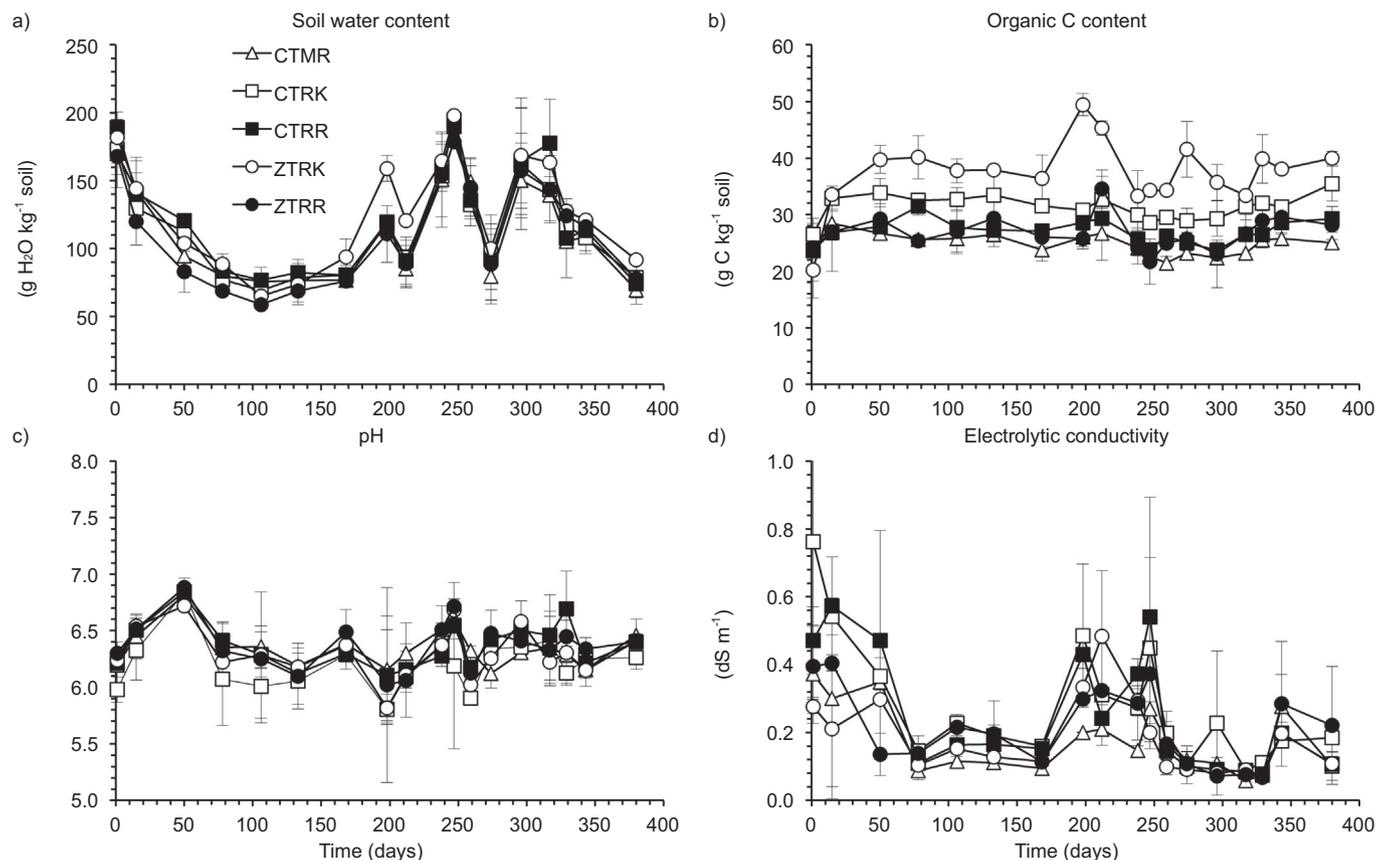
The alpha diversity, i.e. Shannon, Chao1 and Simpson index, were calculated using the rarefied OTU-biom (Shannon, 1948; Simpson, 1949; Chao, 1984). Good's coverage was calculated with the biom table within QIIME (Good, 1953; Caporaso et al., 2010b). All statistical analyses were done in R (R Core Team, 2013). A non-parametric test was used to determine the effect of the agricultural practices on the soil characteristics and the bacterial alpha diversity with the non-

parametric t1way test of the WRS2 package (A collection of robust statistical methods) (Mair and Wilcox, 2017). The bacterial community structure in the different treatments was explored with a principal component analysis (PCA), while the constrained analysis of principal coordinates (CAP) was used to explore the effect of soil characteristics on the bacterial community structure. A permutational multivariate analysis of variance (perMANOVA) using the distance matrices test (usage adonis, method Bray-Curtis) was used to determine the effect of different agricultural practices and time on the bacterial community structure. Heatmaps were constructed with the pheatmap package (Kolde, 2015). Spearman correlation coefficients between the soil characteristics and the relative abundance of the different bacterial communities were calculated in the vegan package in R (Oksanen et al., 2017). A possible effect of soil characteristics (pH, EC, and organic C,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  content) and treatment on the relative abundance of the different bacterial groups was explored with the random Forest algorithm (Breiman et al., 2015). The best subset of environmental variables with maximum (rank) correlation with community dissimilarities (bioenv) was used to determine which soil characteristics (pH, EC, and organic C,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  content) best explained the bacterial community structure. The PCA, CAP, adonis and bioenv tests were done with the vegan package (Oksanen et al., 2017).

## 3. Results

### 3.1. Soil characteristics

The water content decreased from November onwards when the rainy season ended until March (Fig. 2a, Fig. S2). It remained < 100



**Fig. 2.** a) Water content ( $\text{g kg}^{-1}$ ), b) total organic carbon content ( $\text{g kg}^{-1}$ ), c) pH and d) electrolytic conductivity ( $\text{dS m}^{-1}$ ) in soil with conventional tillage, monoculture maize (*Zea mays* L.) and crop residues removed ( $\Delta$ , CTMR), conventional tillage, maize-wheat (*Triticum aestivum* L.) rotation and crop residues kept ( $\blacksquare$ , CTRK), conventional tillage, maize-wheat rotation and crop residues removed ( $\square$ , CTRR), zero tillage with maize-wheat rotation and crop residues kept ( $\bullet$ , ZTRK) and zero tillage, maize-wheat rotation and crop residues removed ( $\circ$ , ZTRR) sampled 18 times at El Batán between 22 November 2016 and 07 December 2017.

g kg<sup>-1</sup> soil until the end of May, and started to increase again when the rainy season began in June (Fig. 2a). The soil water content increased after the onset of rains in the beginning of June. Afterwards, it showed large fluctuations as a result of erratic rainfall. The water content decreased again towards the end of November when the rainy season stopped (Fig. S2). The water content was not affected by treatment considering the whole year (Table 1). The total organic content showed little variation in the different treatments over time, except in the ZTRK treatment around 22 of June (day 198) (Fig. 2b). The total organic content was highly significantly affected by treatment in the order ZTRK > CTRK > CTRR = ZTRR > CTMR (Table 1). The soil pH was slightly acid and showed some fluctuations in the beginning of the year (Fig. 2c), but was not significantly affected by treatment (Table 1). The EC decreased in the beginning of the experiment, increased again in July and August during the rainy season and decreased again thereafter (Fig. 2d). The EC was not significantly affected by treatment (Table 1).

Concentrations of NH<sub>4</sub><sup>+</sup> did not show a clear pattern during the crop cycle, but fluctuated between > 2 and < 50 g N kg<sup>-1</sup> (Fig. S4a). Concentrations of NO<sub>2</sub><sup>-</sup> were low (< 0.6 mg kg<sup>-1</sup> dry soil) and from April onwards no NO<sub>2</sub><sup>-</sup> was detected in soil (Fig. S4b). Concentrations of NO<sub>3</sub><sup>-</sup> gradually increased until June and fluctuated sharply thereafter and little or no NO<sub>3</sub><sup>-</sup> was detected in soil between August and October (Fig. S4c). Concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were not significantly affected by treatment (Table 1).

### 3.2. Changes in the bacterial community structure over time

Overall 2,078,379 bacterial sequences were retrieved from the soil, which represented 33,618 OTUs. The rarefaction curves showed that including more sequences in the study would only yield a limited number of more OTUs (Fig. S5). The Good's coverage (mean of all samples 81%), Chao1 estimator, and the Shannon and Simpson diversity indices were highly significantly affected by time of sampling ( $p < .001$ ) and Chao1 estimator by treatment ( $p < .001$ ) (Fig. S6).

Proteobacteria were the most abundant bacterial phylum (relative abundance 34.84 ± 9.99%, mean of all sampling days and treatments), followed by Actinobacteria (21.55 ± 4.44%) and Acidobacteria (14.14 ± 3.64%) (Fig. 3). Some bacterial groups showed larger variations over time, but no clear pattern emerged. In the CTMR treatment, for instance, the relative abundance of Proteobacteria was 2.4 times larger on 18-07-2017 (63.95%) than on day 7-02-2017

(26.74 ± 4.64%), while that of Actinobacteria was the opposite, i.e. 10.34% on day 18-07-2017 and 26.31 ± 3.05% on day 7-02-2017.

*Kaistobacter* was the most abundant bacterial genus (relative abundance 3.20 ± 1.50%) in the soil and its relative abundance was evenly distributed during the crop cycle (Fig. 4). *Serratia* (2.90 ± 5.82%) was the second most abundant bacterial genus, but its relative abundance showed great variation in a year, as did that of *Bacillus* (1.88 ± 6.79%) and *Pseudomonas* (1.64 ± 5.09%). The relative abundance of *Serratia* and *Achromobacter* increased towards the end of the crop cycle, while that of *Bacillus* peaked on April the 3rd 2017 and that of *Pseudomonas* on the 18th, 27th July and 8th August 2017.

The perMANOVA analysis showed a highly significant effect of sampling time on the bacterial community structure considering the different phyla (F value = 22.54,  $p < .001$ ) and even more so considering the 50 most abundant bacterial genera (F value = 30.73,  $p < .001$ ) (Table S2). A PCA with all bacterial phyla, the 50 most abundant bacterial genera, all bacterial groups assigned to the level of genera, all bacterial orders, the 50 most abundant OTUs and all OTUs did sometimes separated samples taken on 18 July 2017 from the other sampling days, but no clear pattern related to effect of agriculture practices or other sampling times was observed (Fig. S7).

The relative abundance of *Achromobacter*, *DA101*, Firmicutes, *Methylibium*, Planctomycetes, *Serratia* and Tenericutes decreased highly significantly with increased EC, and that of *Agrobacterium* and Gemmatimonadetes increased ( $p < .0001$ ) (Fig. S8). The relative abundance of Bacteroidetes and *Flavobacterium* increased highly significantly with increased soil total organic C content ( $p < .0001$ ). The relative abundance of *Amycolatopsis* and *Opitutus* decreased highly significantly with increased water content, and that of *Achromobacter*, *Kaistobacter*, Proteobacteria and *Pseudomonas* increased ( $p < .0001$ ). The relative abundance of a large number of bacterial groups was affected highly significantly with the concentration of NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> in soil.

The best model considering the bacterial phyla included the NO<sub>2</sub><sup>-</sup> content as parameter in all treatments, except in the ZTRK treatment, and the NO<sub>3</sub><sup>-</sup> content in all treatments considering the 50 most abundant bacterial genera (Table S3). More than 30% of the variation in the relative abundance of Chloroflexi, Cyanobacteria and Gemmatimonadetes could be explained by the random Forest algorithm (considering all soil characteristics) and > 40% of *Methylibium* and *Rhodoplanes* (Fig. S8).

**Table 1**  
Characteristics of soil at CIMMYT's El Batán in Texcoco (Mexico).

Code <sup>a</sup>	Crop rotation	Tillage	Residue management	N fertilizer (kg N ha <sup>-1</sup> )	pH	EC <sup>b</sup> (dS m <sup>-1</sup> )	WC <sup>c</sup> (g kg <sup>-1</sup> soil)	Total C	NH <sub>4</sub> <sup>+</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>
CTMR	MM <sup>d</sup>	Conventional tillage	Residue removed	150	6.3 <sup>e</sup> A <sup>f</sup>	0.176 A	114 A	24.8 D	14.3 A	0.08 A	13.5 A
CTRR	MW <sup>g</sup>	Conventional tillage	Residue removed	150	6.4 A	0.250 A	122 A	26.9 C	13.6 A	0.11 A	21.5 A
CTRK	MW	Conventional tillage	Residue retained	150	6.2 A	0.278 A	117 A	31.3 B	14.4 A	0.09 A	24.9 A
ZTRR	MW	Zero tillage	Residue removed	150	6.4 A	0.215 A	113 A	26.7 C	15.9 A	0.09 A	20.8 A
ZTRK	MW	Zero tillage	Residue retained	150	6.3 A	0.185 A	128 A	37.3 A	14.6 A	0.10 A	14.6 A
F value					2.33	2.31	0.76	61.06	0.03	0.07	1.32
p value <sup>h</sup>					0.068	0.071	0.556	< 0.001	0.998	0.991	0.273

<sup>a</sup> CTMR: soil with conventional tillage, monoculture maize (*Zea mays* L.) and crop residues removed, CTRK: conventional tillage, maize-wheat (*Triticum aestivum* L.) rotation and residues kept, CTRR: conventional tillage, maize-wheat rotation and crop residues removed, ZTRK: zero tillage with maize-wheat rotation and crop residues kept and ZTRR: zero tillage, maize-wheat rotation and crop residues removed.

<sup>b</sup> EC: electrolytic conductivity.

<sup>c</sup> WC: Water content.

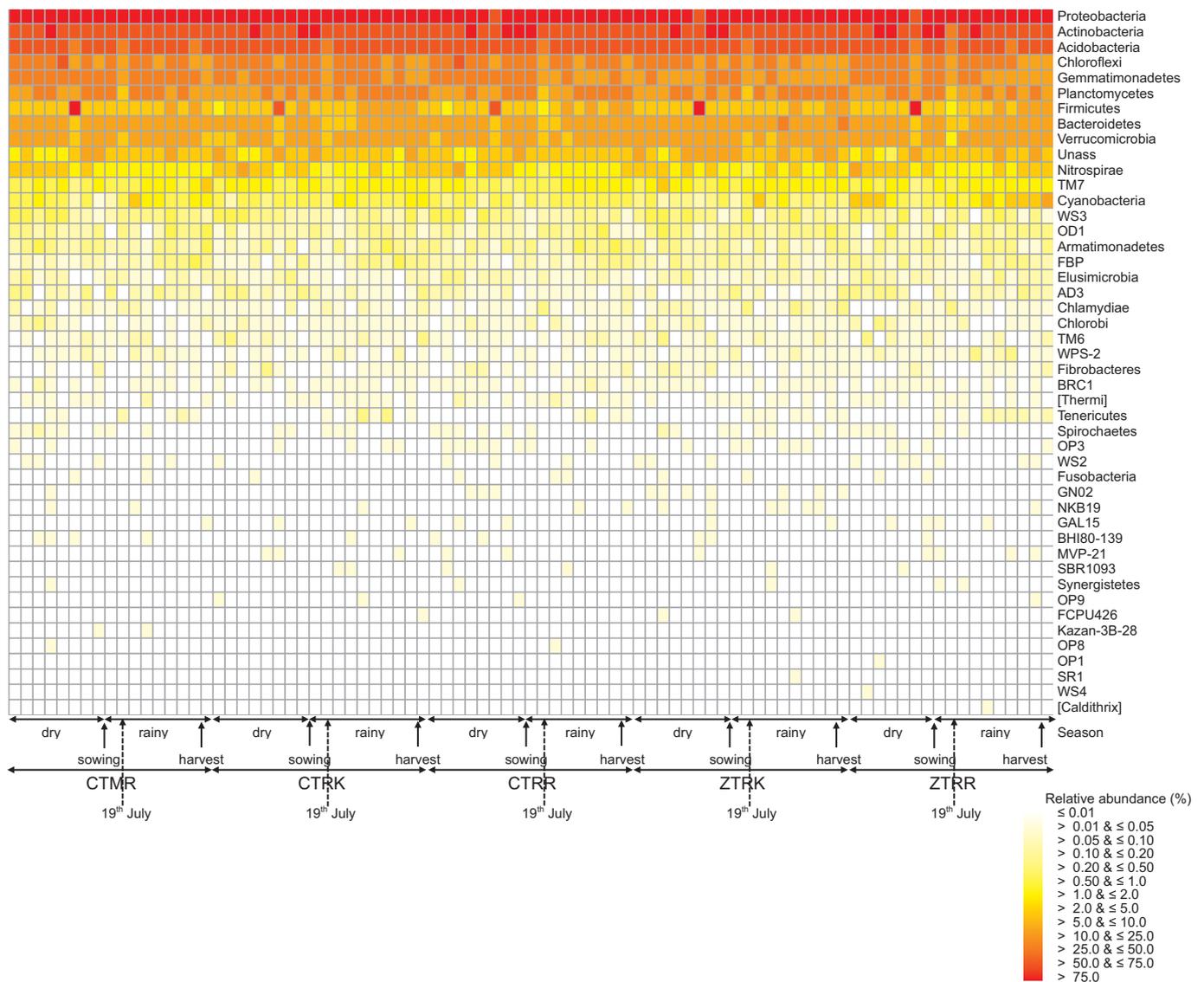
<sup>d</sup> Maize (*Zea mays* L.) monoculture.

<sup>e</sup> Mean of two sampling plots and 18 sampling days.

<sup>f</sup> Values with the same capital letter are not significantly different between the treatments, i.e. within the column ( $p < .05$ ), non-parametric lincon analysis in R library WRS2.

<sup>g</sup> MW Maize wheat (*Triticum aestivum* L.) crop rotation.

<sup>h</sup> Non parametric analysis T1way analysis in R library WRS2.



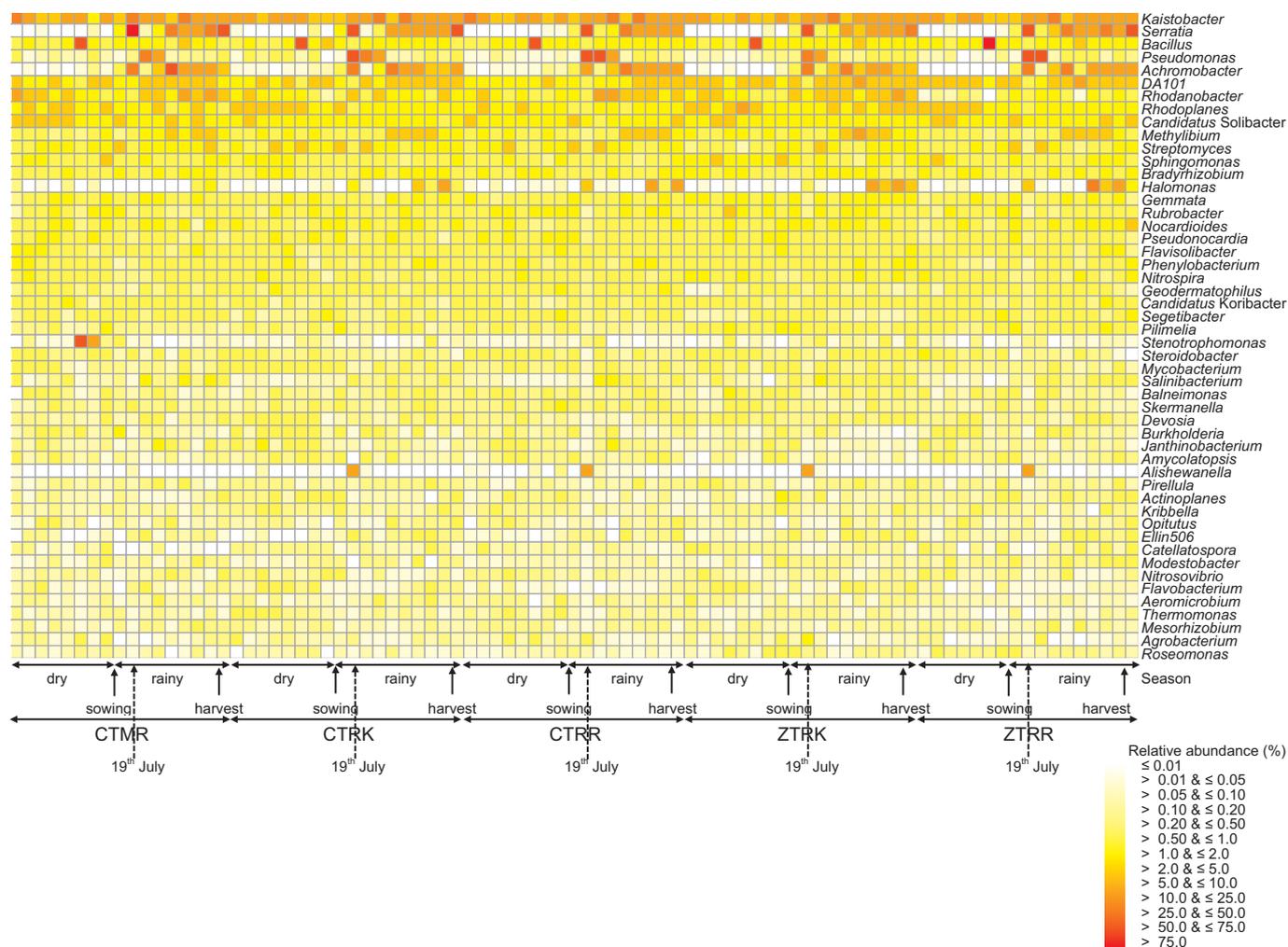
**Fig. 3.** Heatmap with the relative abundance of the different bacterial phyla in soil with conventional tillage, monoculture maize (*Zea mays* L.) and crop residues removed (CTMR), conventional tillage, maize-wheat (*Triticum aestivum* L.) rotation and crop residues kept (CTRK), conventional tillage, maize-wheat rotation and crop residues removed (CTRR), zero tillage with maize-wheat rotation and crop residues kept (ZTRK) and zero tillage, maize-wheat rotation and crop residues removed (ZTRR) sampled 18 times at El Batán between 22 November 2016 and 07 December 2017.

The CAP analysis with the bacterial phyla and the soil characteristics separated the soil sampled on the 18th of July clearly from the other sampling days, but the model was not significant ( $F = 1.50$ ,  $p = .058$ ) (Fig. 5). Proteobacteria (mostly Burkholderiales, Pseudomonadales and Xanthomonadales) were enriched in the wetter soil sampled on the 18th of July (Fig. 3). Some of the soil samples taken at the beginning of the crop cycle were generally characterized by a larger relative abundance of Acidobacteria, Chloroflexi and Gemmatimonadetes, and others by that of Actinobacteria (mostly Actinomycetales) and Firmicutes (mostly Bacillales). The CAP analysis considering the 50 most abundant genera and the soil characteristics, i.e. pH, WC, EC, organic C content, and concentration of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ , was highly significant ( $F = 5.79$ ,  $p < .001$ ) and showed a clear effect of sampling time (Fig. 6). The separation was determined by the relative abundance of mostly six genera, i.e. *Achromobacter*, *Bacillus*, *Halomonas*, *Kaistobacter*, *Pseudomonas* and *Serratia*, and the soil characteristics. Sampling between 22 November 2016 and 22 June 2017 had a higher relative abundance of *Bacillus*, and total N and mineral N content. The bacterial community in the soil sampled on 18 July 2017 had a larger

relative abundance of *Serratia* and *Achromobacter* (Fig. 4), but the soil water content and the relative abundance of *Kaistobacter* and *Pseudomonas* was also larger than in the soil sampled before the 18 of July 2017 and after 8 of August 2017. The soil sampled on July 27 and 8 August 2017 was also characterized by a larger soil water content and relative abundance of *Kaistobacter* and *Pseudomonas* as in the soil sampled on 18 July 2017, but the relative abundance of *Serratia* and *Achromobacter* was lower. A lower soil water content and relative abundance of *Kaistobacter* and *Pseudomonas* characterized the soil sampled after August 8, 2017, and especially on August 23, 2017, but the relative abundance of *Serratia* and *Achromobacter* was higher again.

### 3.3. The bacterial community structure as affected by agricultural practices

The perMANOVA analysis indicated that treatment had a significant effect on the bacterial community structure considering all bacterial groups assigned to the level of genus ( $F$  value = 2.66,  $p < .001$ ) and OTUs ( $F$  value = 4.20,  $p < .001$ ), but less than the effect of time of sampling ( $F$  value = 21.99,  $p < .001$  and  $F = 7.83$ ,  $p < .001$ ,



**Fig. 4.** Heatmap with the relative abundance of the 50 most abundant bacterial genera in soil with conventional tillage, monoculture maize (*Zea mays* L.) and crop residues removed (CTMR), conventional tillage, maize-wheat (*Triticum aestivum* L.) rotation and residues kept (CTRK), conventional tillage, maize-wheat rotation and crop residues removed (CTRR), zero tillage with maize-wheat rotation and crop residues kept (ZTRK) and zero tillage, maize-wheat rotation and crop residues removed (ZTRR) sampled 18 times at El Batán between 22 November 2016 and 07 December 2017. Clustering was done with Boolean values using the library FactoMineR in R.

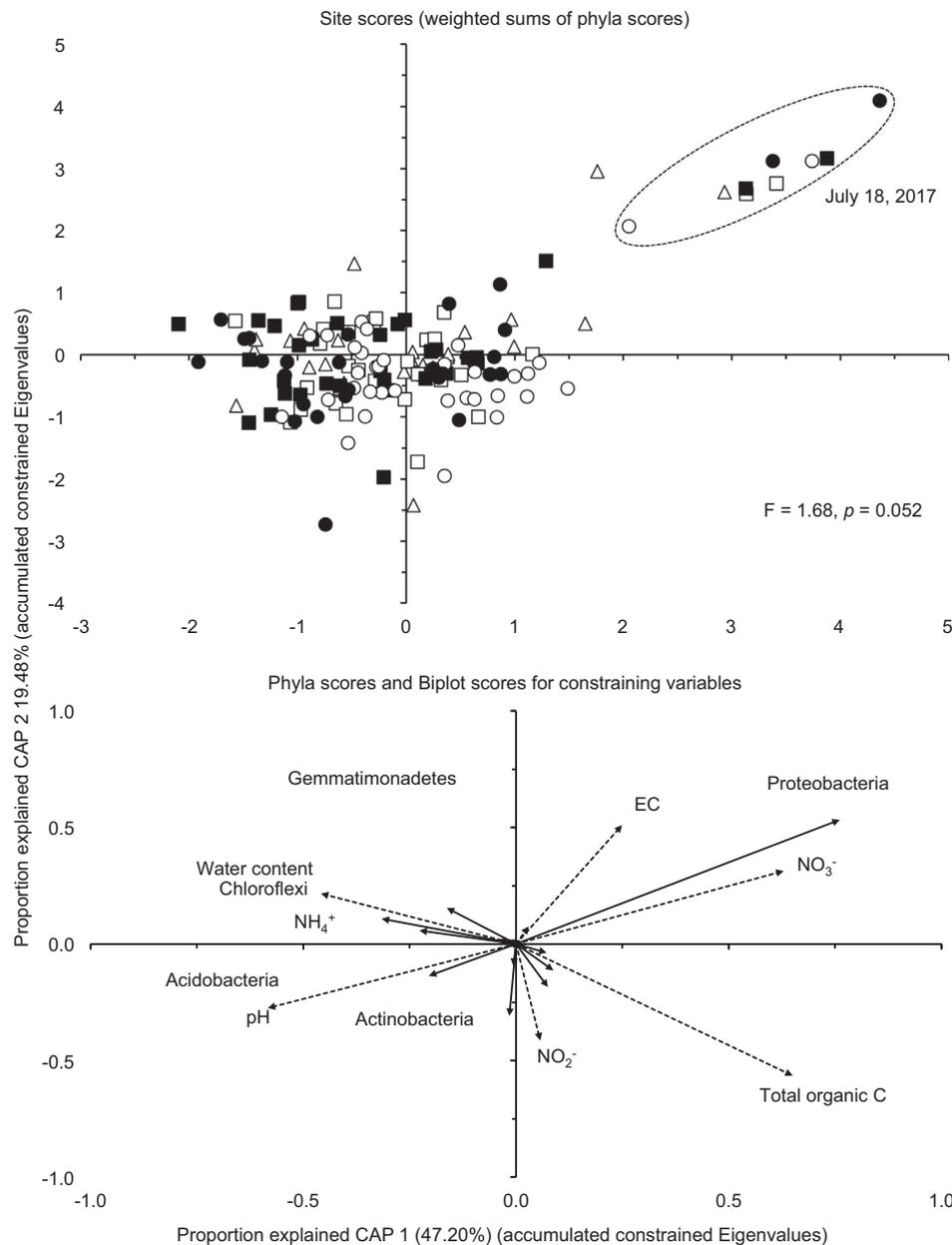
respectively) (Table S2). Tillage and crop residue management had a highly significant effect on the bacterial community considering all bacterial groups assigned to the level of genus and OTUs, but not when considering the bacterial phyla ( $p \leq .007$ ). Crop rotation had a highly significant effect on the bacterial community structure considering all OTUs ( $p = .008$ ).

The PCA considering the bacterial phyla, orders, the 50 most abundant genera, all bacterial groups assigned to the level of genus, the 50 most abundant OTUs and all OTUs did not clearly separate the different treatments from each other as changes over time masked treatment differences (Fig. S7). The CAP analysis did not separate the different treatments on the different sampling days (Data not shown). Additionally, CAP analysis did not separate the different treatments when averaging the relative abundance of the bacterial phyla and the 50 most abundant genera and the soil characteristics and the model was not significant ( $F = 1.71$  and  $p = .177$  for the phyla and 1.43 and  $p = .234$  for genera) (Fig. S9).

## 4. Discussion

### 4.1. Soil characteristics

Soil management practices, such as monoculture or crop rotation, retention or removal of residues, and tillage or no-tillage, have a large effect on soil organic C (D'Acunto et al., 2018). Tillage brings the crop residue in direct contact with the soil microorganisms so that it degrades faster than in the no-till practices where the crop residue remains on the soil surface (Valboa et al., 2015). Additionally, tillage breaks up soil aggregates that protect organic material from bacterial degradation (Schmidt et al., 2011). Incorporation of crop residue through tillage decreases soil organic matter faster through mineralization than in the zero tillage treatments (Upton et al., 2019). Moreover, conventional tillage reduced yield compared to zero tillage with residue retention (Verhulst et al., 2011a), resulting in lower organic material input. The lower soil organic matter in conventional tillage with monoculture compared to crop rotation could also be related to the lower organic matter input with monoculture, since combined yields of maize and wheat were higher than those of maize monoculture (Govaerts et al., 2005). Additionally, wheat leaves a denser stubble and root system than maize in the treatment where



**Fig. 5.** Constrained analysis of principal coordinates analysis (CAP) with soil characteristics, i.e. water content (WC), total organic carbon content (TOC), pH, electrolytic conductivity (EC), ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) concentration and the bacterial phyla in soil with conventional tillage, monoculture maize (*Zea mays* L.) and crop residues removed ( $\Delta$ , CTMR), conventional tillage, maize-wheat (*Triticum aestivum* L.) rotation and crop residues kept ( $\square$ , CTRK), conventional tillage, maize-wheat rotation and crop residues removed ( $\blacksquare$ , CTRR), zero tillage with maize-wheat rotation and crop residues kept ( $\circ$ , ZTRK) and zero tillage, maize-wheat rotation and crop residues removed ( $\bullet$ , ZTRR) sampled 18 times at El Batán between 22 November 2016 and 07 December 2017.

residue is removed due to the different plant population and root system structure (Weaver, 1926).

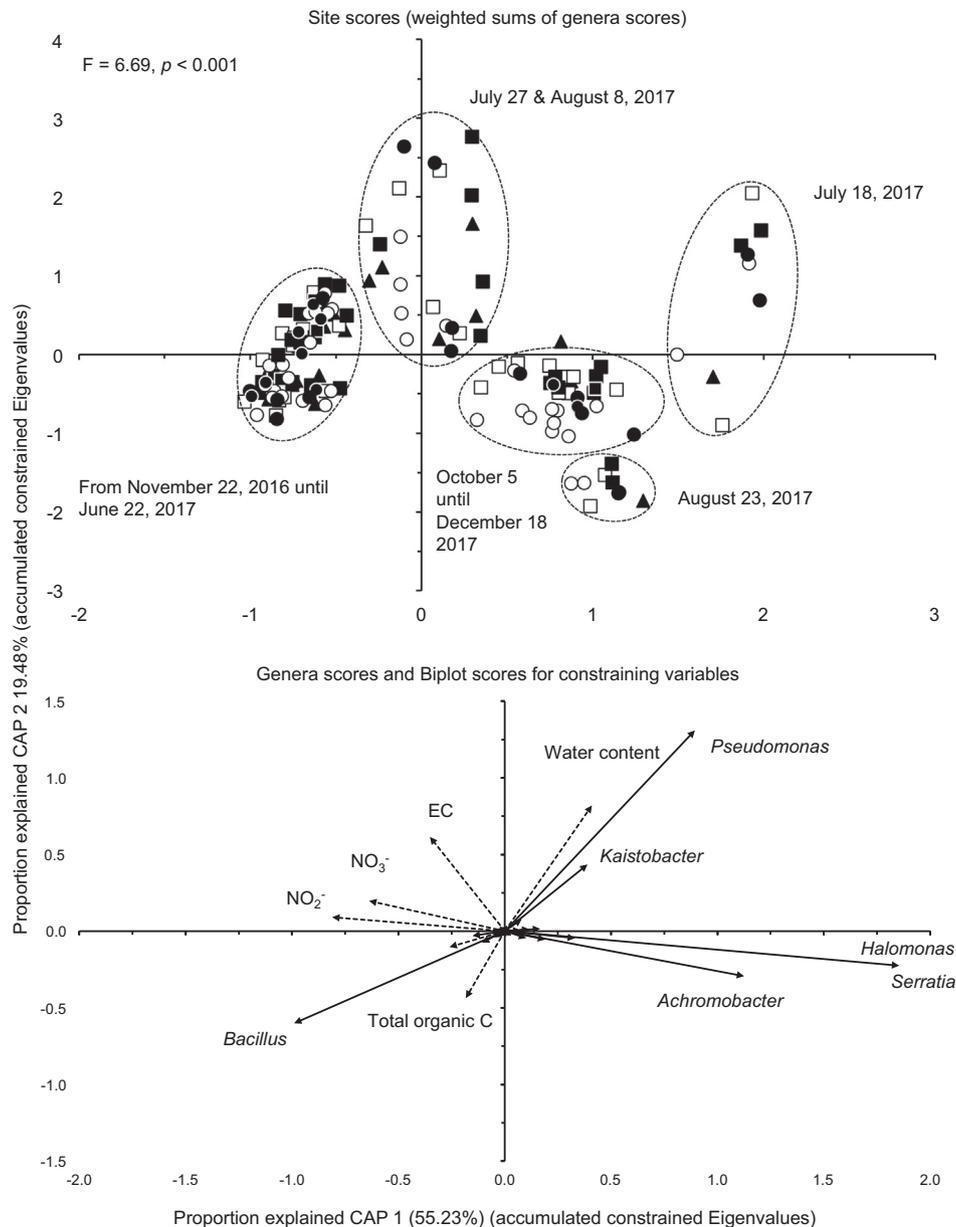
The climate at El Batán is characterized by a prolonged dry period that lasts normally from end of October until mid-May. The soil water content consequently dropped and although some sporadic rainfall occurred in March, April and May the soil water content did not increase notably. Towards the end of May the precipitation increased and consequently the soil water content, most outspoken in the ZTRK. The mulch cover favored water infiltration and reduced evaporation (Govaerts et al., 2009) providing more water for the growing plants. The mean water content, however, was not significantly different between the treatments due to the high variations.

The EC in all treatments increased after sowing and fertilizer application, but decreased after sixty days presumably due to maize development and nutrient uptake (Eyheraguibel et al., 2008). The disruption of soil aggregates did not only release C and N, but also minerals and the incorporation of crop residues further increases the EC (Abdollahi and Munkholm, 2014; Shahbaz et al., 2017). This might explain why the EC was higher in CTRK compared to CTMR or ZTRK.

The nitrate and ammonia content also decreased sixty to seventy days after fertilizer application, as a combination of plant nitrogen uptake, nitrate leaching and/or denitrification (Eyheraguibel et al., 2008; Ward and Jensen, 2014).

#### 4.2. Changes in the bacterial community structure through time

Changes in the bacterial community structure during the crop cycle were larger than the differences in the bacterial community structure as a result of the different agricultural practices applied. Consequently, factors that fluctuated over time, e.g. WC, soil mineral N content, temperature, available organic material, were more important than the factors that were different between the soils in the different treatments at a given moment. Hydrolysis of urea applied as fertilizer released  $\text{NH}_4^+$  that was oxidized to  $\text{NO}_2^-$  and  $\text{NO}_3^-$ . The oxidation of  $\text{NH}_4^+$  in an arable soil is instantly by nitrifiers that contribute only marginally to the bacterial community. As such, the high correlation of the bacterial community with the soil mineral N was not with the mineral N itself, but with the processes that generated it, i.e. mineralization of organic



**Fig. 6.** Constrained analysis of principal coordinates analysis (CAP) with soil characteristics, i.e. water content (WC), total organic carbon content (TOC), pH, electrolytic conductivity (EC), ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) concentration and the 50 most abundant bacterial genera. Legends to the figure can be found in Fig. 5.

material and/or release of immobilized N. The amount and C-to-N ratio of available easily decomposable organic material will determine the bacterial community and its activity will determine the mineral N released in the environment (Masunga et al., 2016; Zhou et al., 2017).

Water content was an important factor affecting the bacterial community structure during the crop cycle (Angel et al., 2010; Wang et al., 2019). Medium annual precipitation and directly soil water content exert an important influence on bacterial community. Tripathi et al. (2017) found that structure and function of soil microbiome are affected strongly by climatic conditions (Moreno et al., 2019). Sudden environmental changes, such as rainfall, might enrich bacterial groups that have normally a low relative abundance (Shade et al., 2014; Jiao et al., 2019).

Six bacterial genera, i.e. *Achromobacter*, *Bacillus*, *Halomonas*, *Kaistobacter*, *Pseudomonas* and *Serratia*, mostly defined the changes over time in the different agricultural treatments. Phylotypes belonging to *Bacillus* were enriched when the soil was characterized mostly by low

amounts of inorganic N and dry conditions, i.e. from the end of November until mid-May. Members of *Bacillus* take an active role in biogeochemical cycles of C, N and P and they are able to solubilize and mobilize K, Zn and Fe (Meena et al., 2016). They can grow under low nutrient availability (McSpadden-Gardener, 2004) and can form spores to survive dry soil conditions (Delgado-Baquerizo et al., 2017). The soil water content peaked towards the end of July, which corresponded with an increase in the relative abundance of members of *Kaistobacter* and *Pseudomonas*. An increased soil water content has a positive effect on the relative abundance of *Kaistobacter* and *Pseudomonas* (Moreno-Espindola et al., 2018). The relative abundance of members of *Achromobacter*, *Halomonas* and *Serratia* was higher towards the end of the growing season and the end of the year when mineral N content was low and the water content higher. *Achromobacter* is often enriched in the rhizosphere so its higher relative abundance towards the end of the growing season might be related to root exudation and presence of the plant (Ely and Smets, 2019). *Halomonas* has been reported as an organic

material decomposer with a high C:N ratio in arable soil (De la Cruz-Barrón et al., 2017). Members of *Halomonas* might be enriched by the rhizosphere of the maize plants. Maize plants are characterized by a high C:N ratio so it can be assumed that the root exudates might also have a high C:N ratio. However, their relative abundance (mean of all sampling days) was lower in the treatments where the crop residue was kept in the field than in the treatments where it was removed. As such, another factor, such as lower nutrient content due to the growing plants, or a combination of different factors might have enriched *Halomonas* towards the end of crop growth. *Serratia* produces auxin-like molecules, fix N<sub>2</sub> as free living bacteria, solubilize phosphate and have a high potential as plant growth promoting bacteria capable to establish a positive interaction with maize (Ludueña et al., 2018; Martínez et al., 2018). All these characteristics might explain why the relative abundance of *Serratia* was high at the end of the growing season.

The effect of changing soil conditions on the bacterial community structure during the crop cycle was less clear when the bacterial phyla were considered compared to when the 50 most abundant genera were considered, but some patterns emerged. After the growing season, Acidobacteria, Chloroflexi and Gemmatimonadetes were enriched. Acidobacteria are considered oligotrophs (Hartmann et al., 2015) and their relative abundance was higher when the soil nutrient content was low in the dry season. Chloroflexi are sensitive to changes in soil structure and N content, but can withstand drier soil conditions (Trivedi et al., 2016). Gemmatimonadetes are usually reported as oligotrophs and adapt easily to dry environments (DeBruyn et al., 2011). Later, when the soil dried out even more, Firmicutes and Actinobacteria were enriched. Actinobacteria are drought resistant and have the capability to degrade recalcitrant organic material (Moreno et al., 2019), while some members of the Firmicutes form spores to survive drought (Wolf et al., 2004; Tripathi et al., 2017). When the wet season started and soil water content increased, Proteobacteria were enriched, especially on July 18th. Proteobacteria are versatile with some members described as copiotrophs that will be enriched by maize residue amendment (Caldwell et al., 2015; Bonanomi et al., 2016; Trivedi et al., 2016; Tao et al., 2018)

#### 4.3. Bacterial community structure as affected by agricultural practices

In a previous study, Navarro-Noya et al. (2013) found a significant effect of residue management and tillage on the bacterial community structure, but did not find an effect of crop rotation. Tillage is known to have a strong effect on the microbial community as it breaks up aggregates, brings organic material in close contact with microorganisms and mixes soil layers (Carbonetto et al., 2014). For instance, Sun et al. (2018) reported that tillage not only affected strongly the bacterial and fungal community, but also changed their vertical distribution. Crop residue serves as C substrate for heterotrophs, so crop residue management, i.e. removal or keeping it in the field normally has a strong effect on the bacterial community (Lu et al., 2015). This study confirmed that residue management and tillage had a highly significant effect on the bacterial community structure, but also that crop rotation, i.e. monoculture of maize versus a rotation of maize and wheat, had a significant effect, considering all OTUs. The effect of crop rotation on the bacterial community might be related to the plant rhizosphere and/or difference in the composition of crop residue, i.e. stubble and roots left in the field (e.g. Haichar et al., 2008; Maarastawi et al., 2018).

Glyphosate was used in the zero tillage to control weeds and this might also affect the bacterial community structure. However, Schlatter et al. (2017) reported that the effects of glyphosate on the bacterial community were smaller than that of location, proximity to the roots and cropping systems.

## 5. Conclusion

The rainfall at El Batán showed large fluctuations and consequently

the soil water content was highly variable during the year, but the soil water content was not significantly different between the treatments. The mineral N, mostly nitrate, as a product of soil organic material mineralization and N fertilizer application also showed large changes over the crop cycle as bacterial activity varies with water and easily organic material availability. The bacterial community structure showed large significant variations during the crop cycle correlated to fluctuations in mineral N and water content. The constrained analysis of principal coordinates considering the 50 most abundant bacterial genera and soil characteristics grouped these variations considering principally 6 genera, i.e. *Achromobacter*, *Bacillus*, *Halomonas*, *Kaistobacter*, *Pseudomonas* and *Serratia*, and water, organic C, nitrite and nitrate content, and EC.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2020.103733>.

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## Declaration of competing interest

The authors declare that they have no conflicts of interest.

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