

## IMPACT OF REDUCED TILLAGE ON SOIL CHEMISTRY AND MICROBIAL COMMUNITIES USED AS AN INDICATOR OF FERTILITY IN A SEMI-ARID REGION

Ahmed Kadri <sup>1,\*</sup>, Ahmed Haddad <sup>2</sup>, Mohamed Laarid <sup>1</sup>, Benkhedda Belhaouari <sup>3</sup>

<sup>1</sup> Agronomy Department, University of Abdelhamid Ibn Badis, Mostaganem, Algeria

<sup>2</sup> University Center Nour Bachir, El-Bayadh, Algeria

<sup>3</sup> Biotechnology laboratory applied to agriculture and environmental preservation, Higher School of Agronomy Mostaganem, Algeria



### Abstract

Tillage is a key element for crop success. Conservation tillage (CT) is frequently mentioned as a useful strategy for enhancing soil quality in agriculture. This studied the effect of reduced tillage on the chemical and microbial properties of the soil in a semi-arid region of northern Africa. At the experimental site in Algeria, soil parameters showed differences between conventional tillage (CT) and non-inversion minimum tillage (MT), with a tillage depth. The results show that the effect of reduced tillage is highly significant ( $P$  value  $< 0.001$ ) on chemical elements compared to organic or mineral management. The Shannon index ( $H$ ) indicates that the diversity is very good ( $H > 5$ ) of the 14 bacterial phyla detected. These results suggest a strategy that minimum tillage (MT) with tine tools better than discs. In order to improve the soil properties, its fertility and the yield of chickpea crops.

Keywords: Bio-Indicators, Cultural practice, Minimum tillage, Soil quality.

### 1. INTRODUCTION

The Soil is the support of most terrestrial life forms and remains extremely complex. It is a layer of minerals physically and chemically modified from the bedrock by geological weathering, nutrient cycling, growth and decomposition of biomass. One gram (1g) of soil can contain billions of bacteria and fungi, as well as plant and animal species. It is the primary growth medium for plants, and thus the environmental resource that supports agriculture (Wright and Hons, 2004; Desjardins et al., 2005).

The bioindicators are species that, by their presence, absence or rarity, can inform us about the state of the environment. They are likely to detect disturbances that have occurred even if they are no longer present at the time of sampling (Belhaouari et al., 2017; Belhaouari et al., 2019). Terrestrial's bio-indicators are defined as organisms that allow the identification and quantification of soil disturbances and the resulting impacts, and therefore monitor the sustainability of agricultural production systems (Bispo et al., 2009).

Microbiological and biochemical properties of soil, including microbial activities and biomass, and enzymatic activities, they are generally considered potentially indicators of soil quality and biological fertility, due to their close liaison with soil organic matter (OM) dynamics and nutrient

cycling, so is the sensitivity of the soil disturbances and Changes caused by tillage (Bastida et al., 2008).

Soil microorganisms are regulated by edaphic factors such as salinity (Lozupone and Knight, 2007), pH (Fierer and Jackson, 2006), soil nutrient status (McCaig et al., 2001), oxygen content, and soil structure (Sessitsch et al., 2001). Additionally, human activities have an impact on soil ecosystems, and proven effects of agricultural practices (McCaig et al., 1999).

Numerous studies have been done on how pH affects the microbial populations in the soil (Lauber et al., 2009). Overall community diversity and richness, or richness, evenness and structure, are greatly influenced by pH. This means that community diversity and composition can be mainly described by pH (Fierer and Jackson, 2006).

According to Lal (2004), improving soil quality will increase soil organic carbon (OC) stock. Many studies have suggested Nitrogen (N) mineralization and Carbon (C) are sensitive indicators of soil quality and fertility (Andrews et al., 2004; Gil-Sotres et al., 2005). Considering the availability and composition of nutrients in the soil (such as organic elements C, N, P, and K), whether in terms of mineralization or direct fertilization, is a good assessment of soil fertility and quality (Srouf et al., 2020).

Biological parameters related to ecology, such as N mineralization and C, are of crucial role and importance in the reactions that occur during the biogeochemical cycle of soil transformation because these two elements reflect on the microbial activity inside the soil (Gregorich et al., 1997; Filip, 2002).

The release of nutrients available for plant growth requires soil microbial activity. An indicator of biological activity in soil is N mineralization and used as an indicator for plant growth by Nitrogen availability, as It provides an indication of the soil's capacity to release inorganic nitrogen for absorbed by plants (Gregorich et al., 1994; Canali and Benedetti, 2006).

Soil denitrification affects carbon availability, the latter a direct control of soil microbial communities. Henry and co-workers showed that restricting C might result in an increase in N<sub>2</sub>O emissions. They also indicated the type of soil affects the composition of microbial communities and nitrifiers. However, the effects detected were not attributed to the C/N ratio; The scientists came to the conclusion that C is not a main factor determining these characteristics of the microbial populations and as a denitrifier.

Microbial activity and soil biomass as well as enzymatic activities have been shown to be among the early responses to changes caused by disturbance in the type of tillage related to cropping practice (Gregorich et al., 1997).

Tillage prepares soils through mechanical disturbance and exerts a range of negative effects on the soil environment of agricultural lands, causing degeneration and severe disruption of the soil ecosystem. The effects of tillage method and type of fertilizers on crop productivity, as well as the chemical and physical properties of the soil, have been studied extensively, but less so on their impacts on soil biology (Cook and Trlica, 2016). No significant pH differences were observed between the various soil treatments, and these values were similar between the fertilizer treatments. Information on the microbial function of agricultural soils is still scarce. We used a metagenomic approach in this study to compare different soil variations affected by different types of tillage, including conventional tillage (CT) with plowing, and reduced tillage (RT). The latter could notably confer stability within the soil microbial community.

The CT system consists of traditional tillage and discing practices prior to seeding. The degradation of large areas of land around the world is attributed to this type of mechanical practice, in contrast to the minimum tillage (MT) system, which is a means to combat soil degradation.

According to Delmont et al. (2012) and Acosta-Martínez et al. (2010), in globally, esearch in soil metagenomics is expanding, but in the agricultural tropics, they are also still rare. Establishing links between microbial diversity and soil function is critical to our understanding of this interaction, specially in arid regions as well, is still far from being clarified.

## 2. MATERIALS AND METHODS

### 2.1. Description of experimental study

In order to compare soil management treatments and land application on the physico-chemical and biological properties of the soil, the realization of experimental device in randomized complete blocks (RCB) with three repetitions was conducted in the experimental station ARDH EL-BAIDHA, belonging to the department of agronomy of the university of Hassiba Ben-Bouali, Chlef, Algeria. Its geographical coordinates: Latitude 36°10'19.6 "N, Longitude 1°14'56.7 "E and Altitude 100m. It lies on an area of three (03) ha, bounded on the North to the East and West by private properties, and to the South by the road No. 19A.

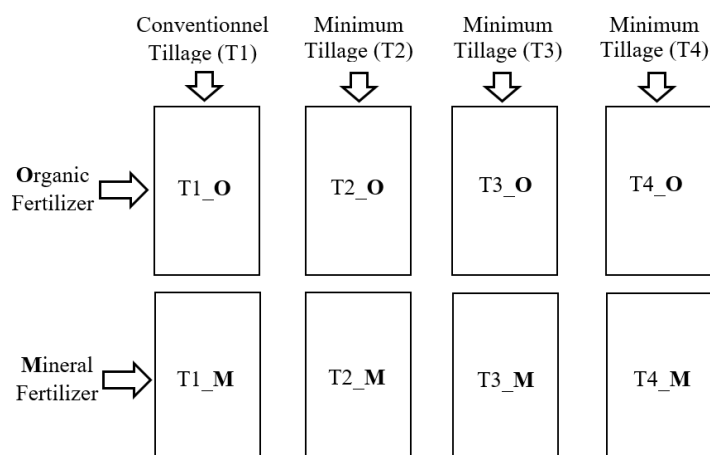
In Algeria, information about soil microbial functions in agriculture is still very scarce, in the present study we compared the cultural practices of agricultural soils cultivated with chickpeas using a metagenomic approach on these microbial communities.

The plot in each block was divided in the direction of width into four (4) microplots, to be able to study the effect of four cultural techniques (T), we opted for the treatments below:

*Table1: Different tools used in cultural practices*

Tillage	Technique	Tools
Conventional	T1	Mouldboard Plough + Cover-Crop + Cultivator
Minimum	T2	Cover-Crop
Minimum	T3	Cover-Crop + Cultivator
Minimum	T4	Cultivator

Each micro plot has dimensions: Length: 15m: Width: 3m, Each micro plot will be divided into two, because we worked on two amendmets: Mineral (M) and organic (O) So that is 8 micro plots (Table 1). On experimental device, the analysis of the soil will be realized before the sowing, to consider as reference of control (witness) as Figure 1.



*Figure 1. Diagram of a block of the experimental set-up*

## 2.2. Soil chemical analysis

Soil samples were collected from the experimental site to a depth of 15 Cm. All soil samples were air-dried at approximately 25°C and then sieved through a 2 mm sieve. The particle size fraction smaller than 2 mm was used for chemical analyses of the soil. They were analyzed in the laboratory using the following methods: electrometric measurement of pH values using a pH meter; the soil was suspended in distilled water (pH water) by a ratio of 1:2.5 (ISO 10390), determination of organic and total carbon (C) (ISO 10390), total nitrogen (N) was measured by the principle of dry combustion method according to ISO 13878, determination of electrical conductivity (EC) by electrometric method according to ISO 11265 and determination of the active lime content CaCO<sub>3</sub>.

Exchangeable cations (Ca, Mg, K, Na) are determined by atomic absorption using an ammonium acetate extract.

## 2.3. Sample preparation and DNA extraction

DNA extraction, sequencing and sequence analysis in the laboratory is done after removing the plant remains; By passing it through a 2 mm sieve, the soil samples were homogenized before carrying out the analysis (Souza et al., 2014).

The preparation of amplicon libraries and the bioinformatic analysis of the sequences were carried out at the Laboratory of Microbial Ecology of the IRDA (Quebec, QC, Canada). The Genetic Analysis Platform of the Institute of Integrative Biology and Systems (IBIS) at Université Laval (Quebec, QC, Canada) carried out a 2x300 bp Illumina MiSeq sequencing.

Samples submitted to the IRDA LEM metagenomic analysis service were prepared and extracted using the FastDNA Spin kit for Soil (MP Biomedicals, Solon, OH, USA). The quality and quantity of the extracted genomic DNAs were determined spectrophotometrically with measurements of absorbance at 260 nm and 280 nm and A<sub>260</sub>/A<sub>280</sub> ratio.

The total number of prokaryotes was calculated using the quantitative polymerase chain reaction (qPCR) method and primers eub338/eub518 (Fierer et al., 2005). Detections were performed in two replicates on a CFX96 instrument (Biorad, Hercules, CA, USA) with SYBR green qPCR mix (Qiagen, Toronto, ON, Canada). The detection system was developed over a 4 LOG detection range. (Total bacteria system efficiency: 89.1%, r<sup>2</sup>=0.99)

Results are expressed in amplification units (AU) per gram of dry soil. It is important to note that several copies of the targeted genes can be detected within the same organism and in variable numbers between organisms during the quantification process.

Metagenomic analyses assessed prokaryotic diversity (bacteria and archaea). Amplification of the 16S rRNA V4 region of prokaryotes was performed using the region-specific primer sequences described by Parada et al. (2016); Apprill et al. (2015), and using two-step PCR approach (a.e dual-indexed PCR approach) designed for analysis with using a Illumina MiSeq high-throughput platform for sequencing. In a paired-end format of 300 base reads the Amplicon libraries were sequenced, i.e., 2 x 300 base pairs on each side of the DNA strand on Illumina MiSeq at the Institute of Integrative Biology and Systems (IBIS) Genomic Analysis Platform at University Laval, Canada.

## 2.4. Bioinformatics and biostatistical processing

Bioinformatics processing of the sequences was performed on the IRDA LEM bioinformatics platform and involved different processing strategies (Qiime2 (Bolyen et al., 2018) and R (R Core Team project 2014)) including quality validation steps, baselines and indexes for measuring

microbial richness, and microbial diversity comparison measures. The Greengenes 13.8 baseline was used for taxonomic assignment of identified genetic variants (DeSantis et al., 2006).

For statistical analyses, data were analyzed using Originpro19 software for Windows. We studied the influence of the following factors: Land tillage technique with or without plowing, the mineral or organic fertilizer bring on microbial communities in soil. The diversity of the Shannon index (H) of the phyla observed in the soil treatments was analyzed with the use of a generalized linear mixed model.

The model estimated the fixed effects of tillage, fertilizer application type, and the interaction effects of tillage with fertilizer and the random effects of replications. Means were separated using the Tukey model at the 0.05 significant level.

### 3. RESULTS AND DISCUSSIONS

#### 3.1. Soil chemical analyses

In this study, the results of chemical analyses presented in Table 2, in the form of Correlation Matrix (Pearson (n)). The effect of the results of analyses between them present in this table also differences between tillage practices.

The elements N, P, K and C having a significant effect on the other elements available in the soil by a percentage reverse of 60% to the averages, more than the main minerals increases the others decreased and vice versa.

Table 2: Correlation matrix (Pearson(n))

K	1										
Na	-0.738	1									
Mg	-0.738	0.748	1								
Ca	-0.158	0.473	0.462	1							
P	0.8	-0.875	-0.664	-0.387	1						
N	0.699	-0.477	-0.569	0.129	0.619	1					
pH	-0.686	0.785	0.373	0.0369	-0.846	-0.509	1				
C	0.405	-0.457	-0.681	-0.481	0.451	0.515	-0.052	1			
CaCO <sub>3</sub>	0.015	-0.25	-0.121	-0.251	0.11	0.264	-0.205	-0.066	1		
EC	-0.561	0.877	0.852	0.523	-0.655	-0.428	0.427	-0.688	-0.133	1	
OM	0.427	0.092	-0.159	0.611	0.085	0.731	-0.177	0.164	0.025	0.122	1
	K	Na	Mg	Ca	P	N	pH	C	CaCO <sub>3</sub>	EC	OM

Existing relationships between the variables taken two by two. From this figure, we can see that P is very good and correlated with K and N, as well as pH with Na. The EC well correlated with Na and Mg and less with pH, however, is not correlated with any other element. CaCO<sub>3</sub> and pH are not correlated with K and Ca respectively. OM is positively correlated with all other elements except Mg and pH. Na and C correlated with most of the other remaining elements.

Energy for microbial decomposition requires sources of C. It is known that minimal work increases the amount of organic matter in the soil (Silva et al., 2014). Also, in our study, total C and total N contents in the soil were higher by 20.85% and 0.164 respectively in minimum tillage (T4\_O) compared to the witness.

The projection of the 9 groups of chemical elements analyzed to the tillage techniques presented in the figure. Given the number of indications of these elements, it appears that there is a discrimination along the axes between the types of tillage studied with the nature of the fertilizer performed.

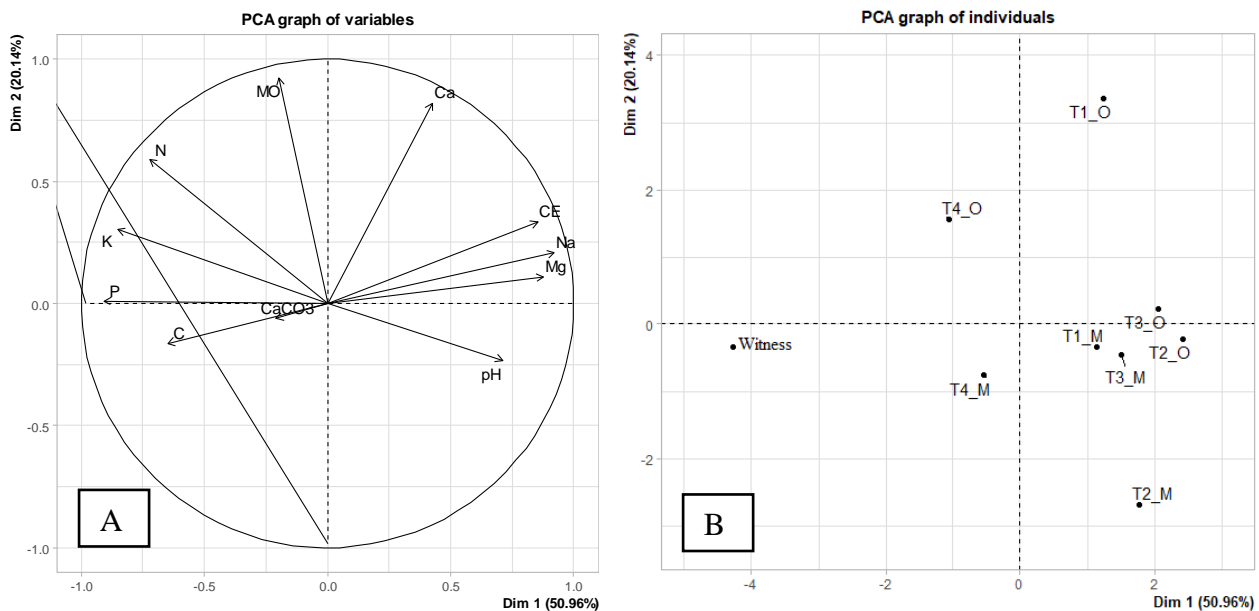


Figure 2. Coordination of principal coordinates (PcoA) of soil chemical analyses.

The correlation circle established between the principal components (Figure 2.A) shows the clear opposition between the groups of variables except for CaCO<sub>3</sub> and C. We can have that the soil pH opposes the elements N, P, K and OM, the latter opposes CaCO<sub>3</sub> and C. While the elements Na, Mg, CE, are well correlated with each other, so they form the same group and show a similar behavior, as well as for CaCO<sub>3</sub> and C, they are well correlated with each other. On the other hand form a group, they have a similar behavior.

On the PCA graph of individuals (Figure 2.B), the conventional tillage with organic amendment (T1\_O) as well as the reduced one with cultivator (T4\_O) induces a major modification to some chemical elements increasing in comparison to the witness. On the other hand, the other tillage management seems to be similar to each other and causes a variation in decrease of elements compared to the witness, without neglecting the effect of fertilizer. This was explained by the decrease of P from 0.53 meq/100g in the witness to less than 0.38 meq/100g in techniques T1\_O, T4\_O and T4\_M. It can be seen that tillage with tines causes a change in the chemical elements that increased with the use of organic fertilizer. On the other hand, these elements decrease with the mineral amendment in the minimum tillage using disc tools.

This work has revealed the decrease of nitrogen rate N from 159 meq/100g to 130 meq/100g as minimum value in minimum tillage with disc tools or using mineral fertilizer, although the crop is chickpea, leguminous fixing soil nitrogen. These results are probably due to the effect of the disc tools in comparison with those with teeth, using minimum tillage. This agrees well with the results obtained by Mariela et al. (2016) and Pérez-Piqueres et al. (2017). The interaction effect of tillage and fertilization density on the chemical properties of the soil is illustrated in Table 3.



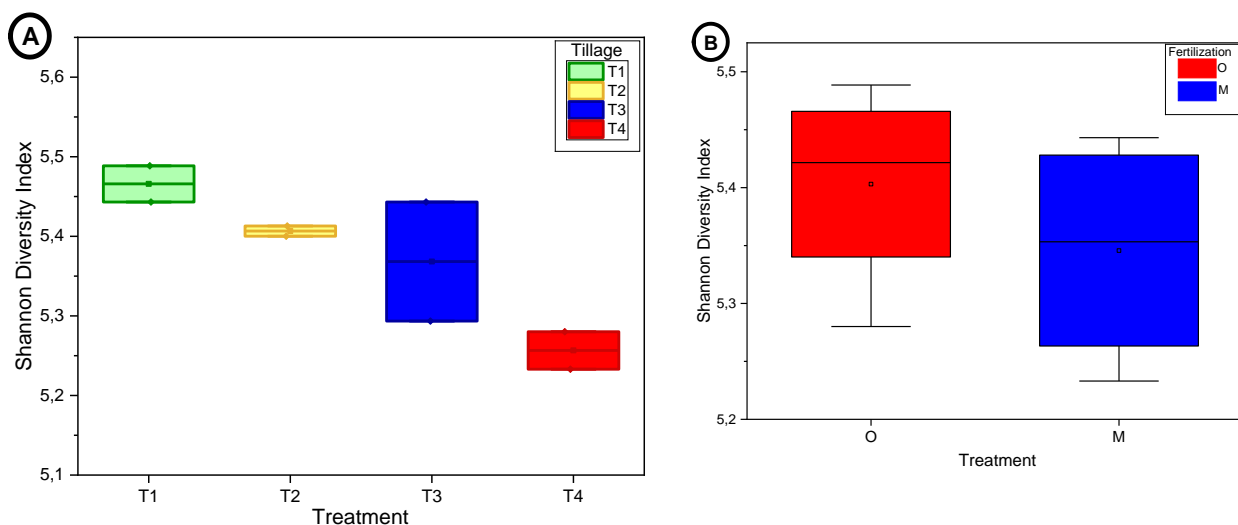
**Table 3. Effect of soil management on fertility indicators.**

Indicator	P value*					
	K	P	N	pH	C	MO
Tillage	1.88E-45	3.10E-50	6.56E-26	3.70E-21	8.89E-42	7.96E-284
Fertilization	3.28E-21	4.25E-34	1.12E-21	3E-4	3.43E-20	2.86E-283
Interaction Tillage : Fertilization	1.39E-31	1.16E-39	1.91E-22	8.62E-10	1.13E-35	3.49E-281

In this study, the results show a very highly significant effect of tillage and fertilization (\*P value 0.001), as well as the interaction of tillage and fertilization on the results of chemical analysis in the different types of tilled plots. The same results were observed by Melero et al. (2008), Madejón et al. (2009), and Panettieri et al. (2020).

### 3.2. Impact of study factors on diversity

The Shannon diversity index of microbial richness and the comparison matrices were determined with the normalized data based on the sequences per sample (figure 3). We notice from the figures (A and B) that the difference in the main methods of tillage and the types of fertilizers used differently affected the diversity of bacterial communities..



**Figure 3. Shannon diversity index of soil management**

This study demonstrates that tillage is the main influencing factor leading to the biodiversity of bacterial communities in different treated soil plots. Furthermore, a shift in diversity to the tillage techniques T3 followed by T4 and T1 which had the highest diversity compared to the other techniques (Figure 3. A). The conventional tillage presents the highest Shannon index, while the minimum tillage T4 presented the lowest index. In addition, the organic fertilizer treatment increases the Shannon diversity compared to the mineral one (Figure.3. B). we notice that this index is higher than 5 (5) which indicates a very high diversity. Thus, the effect of tillage was more important than that of fertilizer.

It can be considered that in this study on microbial communities associated with tillage and fertility treatments that the Shannon diversity index different from one tillage to another. Although this index evolved at minimum tillage, but more important at tillage because of disturbance undergo the soil. The conventional tillage improved the soil structure, (aerate, increase the structural macroporosity of the soil to facilitate the penetration of plant roots, Accumulation and conservation of water, burying fertilizers, plant debris and amendments). This is in agreement with the results obtained by Vasconcellos et al. (2013), Srour et al. (2020). Hence, environmental groups, including mycoparasites, arbuscular mycorrhizal, and filamentous fungi, were favored in no-tilled soils, while plant pathogens and fungal tracers dominated in tilled soils. As evidenced by a number of research, the conventional tillage and fertilizer management transferred communities to rapidly expanding rivals as shown in several studies by Chagnon et al. (2000), Anderson (2003) and Sekaran et al. (2020).

### 3.3. Effects of tillage on bacterial communities

For the study of the effect of soil management on the microbial community, over 26,000 valid reads were obtained for each replicate treatment. The median sequence length of each read was 255 bp.

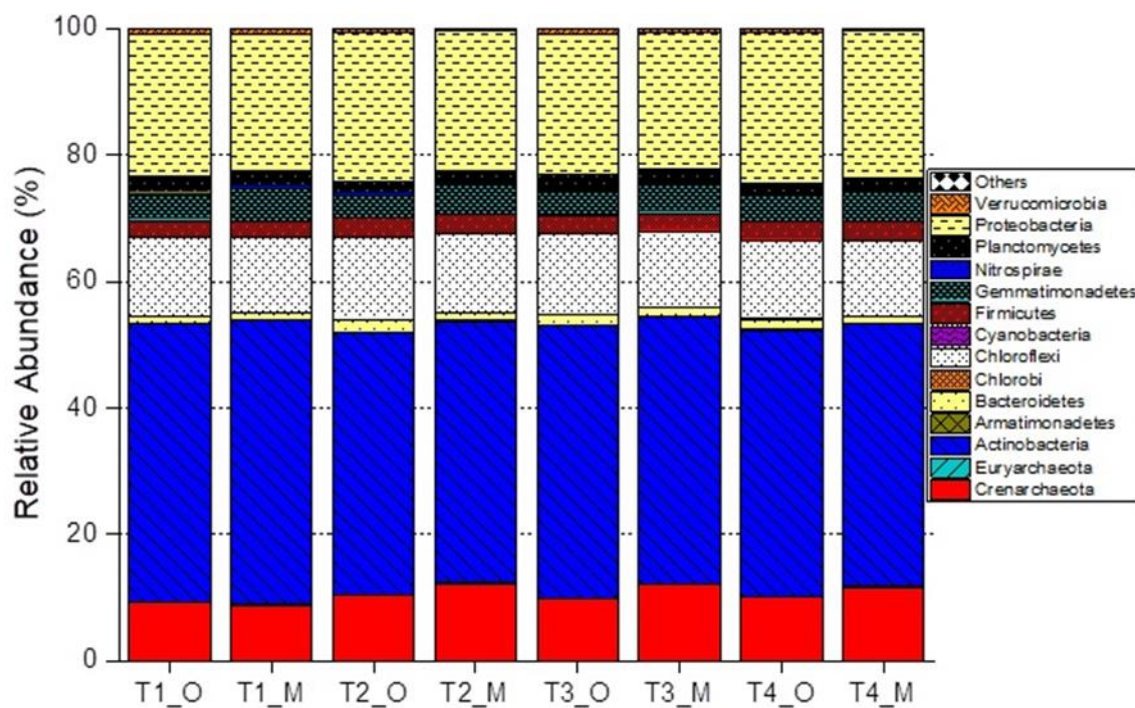


Figure 4. Bacterial phyla's relative abundance under various tillage and fertilizer systems.

The total bacterial community has detected using 95% identity as a threshold in the samples analyzed, the number of phyla determined in the tillage management was 14. The effect of tillage type associated with fertilizer type on the divergence in relative abundance was observed illustrated by relative abundance of each phylum (Figure 4).

The present finding showed a clear dominance of the phylum belonging to *Actinobacteria*, *Proteobacteria*, was more abundant, followed by *Chloroflexi* under the different treatments performed. The dominant phyla were *Actinobacteria* (44.93%), *Proteobacteria* (22.37%) and



*Chloroflexi* (12.47%) under conventional tillage management with ploughing (T1), on the other hand the relative abundance of *Nitrospirae* and *Verrucomicrobia* was lower in minimum tillage, while the *Chlorobi* were present only in minimum tillage with organic input, which opposed to the abundance of *Cyanobacteria* appear only with mineral input with major soil disturbance.

The structure in bacterial communities presents a very highly significant difference between the samples of conventional tillage or minimum (P value<0.0001).

In Algeria, studies on the meta-genome of bacteria in agricultural soils have not been widely reported. However, no studies were published on the effect of soil preparation techniques on microbial communities. This study on fertility bioindicators in an arid region in northwestern Algeria revealed the presence of phylum *Chlorobi* only on plots treated with organic fertilizer. This was probably due to the use of organic fertilizer that provided this type of bacteria.

The impact of adopting different long-term tillage systems and fertilizers on soil physicochemical factors and nutrient cycling has been extensively studied and described with a wide range of crops and different soil types around the world (Alvarez and Steinbach, 2009). In the present study, we sought to evaluate the combined effect of certain types of tillage and fertilization on the chemical properties and microbial community including bacteria. Specifically, we explored the induced changes in soil fertility bioindicators under two tillage regimes (conventional and minimum) in combination with fertilizer applications (Organic, and Mineral).

Also, this work revealed that soil bacteria are strongly affected by tillage and fertilization mode, According to the results observed by Demoling et al. (2007) and Somenahally et al. (2018). Similarly, including organisms were favored in tilled plots due to the degradation of macro-aggregates caused by soil disturbance.

#### 4. CONCLUSIONS

This study demonstrates that different soil management practices alter soil microbial composition and functional diversity of bacterial communities. We found that disturbance of tillage had the greatest effect on the microbial environment compared to fertilizer additions. Community composition and function varied significantly between conventional and minimal tillage, while differences in response to different fertility treatments were limited. Experimental results indicate that minimal tillering with dental tools is better than tillering with discal tools, but they need more long-term research.

#### 5. ACKNOWLEDGEMENTS

I would like to thank Mr. Omar ZIDOUNE, Architect from MECHERIA, W.NAAMA for supporting me during this research. I particularly thank Ms. Aziza HAMMADI, PhD from the University of BATNA (Algeria), for helping me to write this document. Furthermore, we thank our colleagues, Mr. Mokhtar SAIDI, Mr. Abdelkader ELOUISSI, from University Mustapha Stambouli of Mascara (Algeria).

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