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Assessment of different Agricultural Techniques on Soil Biological Activity of Olive Tree in the Southern Region of Tunisia

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Abstract

The climate change and water shortage have a huge drawback in the agriculture in the dry region. The case of drought was observed in the South of Tunisia in the Olive tree culture. Indeed, this degradation results from an obvious reduction in the soil activity. The plantation of the fig tree in intercalary in with Olive tree the soil plot (P4) and a soil Olive mill wastewater OMW spreading plot (P3) were involved in soil remediation in the Olive tree Field. These plots were compared with untouched soil (P1) and soil planted with Olive tree since 1900(P2). The P3 showed an important Organic Matter (2.14mg/g). However, the Soil fertility measured via Germination Index (GI) revealed an important rate (200.08 %) in P4. In contrast to the soil fertility of that P3 didn't exceed the 60.20%. Correlation analyses between different soil parameters revealed significant trends; especially that observed by the negative correlation between respiration soil activity (Resp) and organic matter (OM) ($r=-0.610$, $p<0.05$). Indeed, a soil CO_2 sequestration may take place. In addition, an important bacterial diversity revealed by Denaturing gradient gel electrophoresis *DGGE molecular technique* was observed in the P3. This result suggested the beneficial effects of OMW spreading on the soil bacterial biodiversity.

Keywords: Olive tree, dry soil fertility, dry soil remediation, Dry Crop culture, DGGE technique.

1. Introduction

In the last years, global climate change has risen the global temperature more than 1°C (Vijayaraghavan *et al*., 2006).This climate change can manually be due to the wind erosion and the soil overexploitation, especially in the dry region. Indeed, the climate change and water shortage have a hard drawback in the agriculture in this region, which is the case of drought observed in the South of Tunisia. The olive tree culture in Tunisia deserves an interest (Nefzaoui, 1991). In fact, the Olive tree is the best adapted to the soil diversity, because it is capable of answering at the needs of reforestation, the fight against the erosion and the soil desertification. However, during the last decade, the olive tree arranges no more water reserves that can be exploited especially during extremely dry periods (Nefzaoui, 1991; Abichou, 2011). Consequently, this degradation results, in fact, from an obvious reduction in the soil's activity. The soil activity is maintained by the contribution of organic matters, soil nutrient sand by soil fauna contribution. One of the important components of the soil biological activity is the one that refers to the microbial activity. Given that microorganisms play the role of catalyst in the soil fertility. It is acknowledged that the soil's microbial activity is related to the ecosystem stability and fertility, and that soil fertility is a major problem in dry regions (Hannachi *et al*., 2014). However, the soil microbial activity is closely related to a cumulative impoverishment of the soil nutrients. Thus, it is necessary to develop the agricultural fertilizing methods for a long-term soil fertility enhancement. Moreover, agricultural irrigation with Olive Mill Wastewater (OMW) effluents was known as a common practice in semi-dry regions (Mekki *et al*. 2006; Angelakis *et al*. 1999). Despite the critical problem of the OMW, it showed an important of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), high contents of organic matter and suspended solids (Dermeche *et al*., 2013; Aggoun *et al*., 2016). In addition, OMW was very efficient for agriculture because this effluent is rich of organic matter and nutrients (Saadi *et al*., 2007; Di Serio *et al*., 2008; Chartzoulakis *et al*., 2010).

The originality of this work does not press for the use of OMW only as a fertilizer. The other Farming technique was also exanimate. We know very well the beneficial effect of spreading OMW on soil, especially in the semi-dry soil that suffered the erosion and the desertification. This study aims at emphasizing the suitable agricultural technique such as the introduction of the Fig Tree in parallel with the spreading of OMW on soil. Indeed, the introduction of the fig tree in intercalary with olive tree can reduce wind erosion and soil evaporation through boosting roughness, in addition to the increase of biological activity due to the increase of the rate of shade plants. In this regard, the soil remained a factor that less studies for a long time in spite of its determining role in the olive culture production. The changes in the physicochemical and biological properties of the soils over crop time (0 to

100 years), type of crop (olive trees and fig trees) and the treatment with OMW spreading were evaluated.

2. Materials and methods

The Objective of this experiment is the study of physicochemical, biological and microbiological soil diversity proprieties in the following plots:
- Plot 1 (P1): plot with untouched soil.
- Plot 2 (P2): plot with degraded soil, soil planted with Olive tree (*Olea europaea* L. *the variety of Zalmati*) each 24 meters during more than 100 years.
- Plot 3 (P3): plot with soil planted with Olive tree (*Olea europaea* L.) each 24 m and spreading with dose of OMW of 200 m^3 during 10 years.
- Plot 4 (P4): plot planted with Olive tree (*Olea europaea* L.) each 24 m and planted with a fig tree (*Ficus carica L, the variety of Zidi*) in the middle of two olive trees. Soil samples were taken at a depth of 15 cm, between two olive trees, except at P4 where it was collected at the shades of the fig tree. Soil cores samples were taken with Hand auger tube *H-4268*. Each sample was corresponded to soil cores collected from four different points.

2.1. Study Zone description

The studied soil plots were located in the region of Chammakh-Zarzis in southern Tunisia, (Southern Tunisia, 33° 36'N, 11° 02'E). The soil texture was: fine sand, 85.9 %, clay 7.7%, coarse sand, 5.7% and silt 0.7% in 0-15 cm of the deep, as described by Abichou (2011).

Regarding the plot treated with OMW: the OMW is pumped from a pit cistern in a tank and brought by a tractor to the field. The OMW was taken from a three-phase discontinuous extraction factory located in the region of Chemmakh-Zarzis. The characteristics of the chemical composition of OMW were in (g/l^{-1}): 105 for Chemical Oxygen Demand (CDO), 55 for Biological Oxygen Demand (BOD), 107 for Organic matter, 11.4 for reducing sugars, 3.9 for Glucose, 5.8 for Phenols, 4.5 for Greasinessmatter, 13.7 for Mineral matter, 1.4 for Nitrogen, 0.32 for Phosphates, 7.5 for Potassium, 0.65 for Magnesium, 1.31 for Sodium, 0.71 for Calcium, and 0.56 for Chlorures. Its humidity was 87.9 %, pH 5.5 and its electrical conductivity was 18.6 (mS/cm^{-1}) (Abichou *et al*., 2017).

2.2. Soil characteristics analysis

The pH and the Electrical Conductivity (EC) of each soil sample were determined with pH meter *XP, pH50 lab* model and Conductivity *inoLab WTW 7110* model, respectively. Total organic carbon (TOC) was determined by the *Walkley–Black* method, and then1.724 to obtain the organic matter (OM) value **(**Walkley, 1947) multiplied the TOC. Total nitrogen soil contents were determined by the Kjeldahl Standard method (APHA *et al*., 1997).

Biological activity in the soil was evaluated by measuring CO_2 evolution in the aerobic condition (Ohlinger, 1995). The soil sample was humidified to

50% of its water holding capacity, and then was incubated at 25°C in the dark. The CO_2 evolved was trapped in NaOH solution and titrated with HCl.

The Soil OMW spreading Phytotoxicity was studied according to the method proposed by (Zucconi *et al*., 1981). This technique consists of making germinate, in petri dish, 10 seeds of tomatoes with the different samples studied, and then the Petri dish were incubated during one week in the dark at 25°C. The Control ground (P1) was used as positive control. After incubation, the reading of the result was determined by counting of the number of the germinated seeds. The length of the roots of seeds having germinated was measured in mm. The germination index ' GI ' was calculated according to the following formula:

GI (%) = [Number of seed germinated*root length]/[Control Number of seed germinated* Control root length]*100.

The Different Soil analysis properties were run in parallel.

2.3. Bacterial Soil analysis

Total genomic DNA was extracted from samples from each ground plot sample, using the commercial Kit FastDNA SPIN Kit for Soil (*MP Biomedicals, Solon, OH, USA*). The region belonging to the 16S rRNA genes corresponding to position 341–907 for Bacteria domain was PCR-amplified at annealing temperatures of 52°C. The amplification reaction was performed according to the taq DNA polymerase protocol (Promega, Madison, WIS, USA). The PCR conditions were as follows: 10 min of initial denaturation at 94°C, 30 cycles at 94°C for 1 min and annealing at 52°C and 56°C for 1 min, 72°C for 3 min, followed by 10 min at 72°C of final primer extension. The DGGE (Denaturing gradient gel electrophoresis) analysis of the PCR products was performed by electrophoresis in a TAE buffer solution (80 mol$L–1 Tris, 2.0 mol$L–1 EDTA, 40 mol$L–1 sodium acetate, pH 7.4) for 5 h at 200 V and 60°C using the DCode TM Universal Mutation Detection System (BioRad, USA). Poly-acrylamide gels 6% (w/v, acrylamide-bisacrylamide37.5:1) were prepared with a denaturing gradient ranging from 30% to 60%, in which the 100% denaturant contained 7 molL–1 urea and 40% v/v formamide.

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Version 20.0). Data are presented as means ± SD. Values were obtained from triplicate determinations and the differences were examined using one-way analysis of variance (ANOVA) followed by the Fischer's LSD (Least Significant Difference) *post hoc* test. Statistical significances of the correlations between data sets were calculated using Pearson's R-values. At least three replicates were performed for each laboratory measurement.

3. Results and Discussion

3.1. Physicochemical properties of soil

The Table 1 revealed the different physicochemical properties of soil in the different studied plots.

Table1. Physicochemical soil plot properties: pH, OM EC, C, N and C/N. LSD (Least Significant Difference) post hoc test. * $p<0.001$ as compared to P1; $^{\alpha}p<0.01$ as compared to P2; $^{\$}$ $p<0.001$ as compared to P3; $^{@}$ $p<0.001$ as compared to P4.

Plot	pH	OM (mg / g)	EC (mS/cm^{-1})	C (mg/g-soil)	N (mg/g-soil)	C/N
P1	8.41±0.02	$2.14\pm0.03^{\alpha@}$	$0.93\pm0.04^{\alpha\$@}$	$1.24\pm0.02^{\alpha@}$	$0.3\pm0^{\alpha\$@}$	$4.13\pm0.06^{\alpha\$@}$
P2	8.54±0.05	$0.84\pm0.03^{*\$@}$	$0.53\pm0.02^{*\$@}$	$0.49\pm0.02^{*\$@}$	$0.17\pm0.001^{*\$@}$	$2.74\pm0.1^{*\$@}$
P3	7.41±1.38	$2.14\pm0.04^{\alpha@}$	$0.61\pm0.01^{*\alpha@}$	$1.24\pm0.02^{\alpha@}$	$0.41\pm0.01^{*\alpha@}$	$3.03\pm0.11^{*\alpha@}$
P4	8.01±0.01	$1.52\pm0.02^{*\alpha\$}$	$1.53\pm0.03^{*\alpha\$}$	$0.88\pm0.01^{*\alpha\$}$	$0.43\pm0.01^{*\alpha\$}$	$2.02\pm0.09^{*\alpha\$}$

An important significant difference in the different parameters ($p<0.01$) was observed between different studied plots. As it can be seen, a basic pH (in the average of 8) was registered in the most studied soils. It is not surprising, in fact the semi-arid soil in the south of Tunisia was known by its basic pH nature (Sahraoui *et al*., 2012). In addition, this soil has an important buffering capacity due to its higher content in bicarbonate that could maintain the soil pH stability (Sahraoui *et al*., 2012). However, a decreasing in the soil pH was observed in the P3 (Plot treated with OMW). Indeed, previous studies suggested that acidic pH of soil is mainly caused by the presence of organic acid in OMW used for soil irrigation (Barbera *et al*., 2013).

Concerning the EC, we note an important increasing of (1.53mS /cm^{-1}) in the P4; these nutrients salts released by soil mineralization (Kavvadias *et al*., 2010) could explain the increase of these salts nutrients in this plot. The weak soil OM content in the P2 plot planted since 1900 revealed a soil degradation status in this Plot compared to the other plots. Both Plots treated with OMW and plot planted with the introduction of Fig intercalary showed an important increasing of soil OM of (2.14mg / g) and (1.52mg / g), respectively. Several studies suggested the beneficial role of OMW soil spreading in the OM and Total carbon contains increment (Mekki *et al*., 2013; Barbera *et al*., 2013). However, the C/N ratio in the treated soils (P3 and P4) showed a decreasing compared to the untouched soil (Table 1). This decrease can be explained by nitrogen rising rate, which affect the kinetics of OM degradation during humification phase (Barbera *et al*., 2013).

3.2. Soil Respiration and Phytotoxicity

Table 2 showed the soil respiration activity and soil phytoxicity defined by CO_2 releases and germination index GI, Respectively. An important respiration soil activity (0.72 mg CO_2/ g 24 h) was registered in y (P4). This result suggested that soil implanted of the Fig tree in intercalary with Olive tree enhance the soil respiration illustrated by an important emission of their contents in carbon in the long term. In contrast, in the plot irrigated with OMW (P3), the soil respiration was low (0.25 mg CO_2/ g 24 h). This result could be explained by the inhibitory effect of the phenolic compounds (Zenjari and Nejmeddine, 2001; Obied *et al*., 2005).

Table 2. Soil Respiration (Resp) and Phytotoxicity (IG).
LSD (Least Significant Difference) *post* test: $^{*}p<0.001$ as compared to P1; $^{\alpha}p<0.01$ as compared to P2; $^{\$}p<0.001$ as compared to P3; $^{@}p<0.001$ as

Plot	IG (%)	Resp (mg CO_2/ g 24 h)
P1	-	$0.24\pm0.01^{\alpha @}$
P2	$101\pm1^{\$ @}$	$0.53\pm0.01^{*\$ @}$
P3	$60.20\pm0.26^{\alpha @}$	$0.25\pm0.01^{\alpha @}$
P4	$200.08\pm0.07^{\alpha \$}$	$0.72\pm0.03^{*\alpha \$}$

Moreover, the inhibitory effect of OMW compared to P4 soil spreading was also observed in the soil phytotoxicity in the P3. Indeed, the value of GI was low (60.20%).The phenolic substances are less easily degradable and their high soil toxic level was reported in previous works (Mekki *et al*., 2007; Buchmann *et al*., 2015). However, the beneficial effect of the introduction of the Fig tree in intercalary with Olive tree was observed in another time by the soil fertility increment (GI: 200.08%).

3.3. Soil parameter correlations

The analysis of correlation of soil parameters revealed significant trends (Table 3). The positive correlation between EC and Total nitrogen (r=0.613, p<0.05) could be explained by the salt nutrients accumulations generated during mineralisation process (Barbera *et al*., 2013). However, the salts nutrients accumulations don't have any inhibitory effect on soil fertility and soil respiration, this observation was concluded by the positive correlation founded between EC and IG and EC and Resp of (r=0.620, p<0.05) and (r=0.600, p<0.05), respectively. Concerning the significant positive correlation observed with IG and Res (r=0.940, p<0.01), it is not surprising. Indeed, the soil CO_2 releasing was generally induced by the microorganism activity, which could closely relate to soil fertility increasing (Barbera *et al*., 2013). However, the negative correlation between the soil's respiration activity (Resp) and OM (r=-0.610, p<0.05) suggested by previous studies which demonstrated that increasing soil OM was a beneficial strategy of (CO_2) sequestering opposite to the global warming increasing (Liu *et al*., 2014; Barton *et al*., 2016).

Table 3. Correlation matrix (Pearson's r values) between the different parameters determined in soils corresponded to different Plots. pH, OM (Organic Matter), EC (Electric Conductivity), C (Total carbon), N (Total azote), IG (Index of germination), Resp (Respiration)
$^{*}p<0.05$ - $^{**}p<0.01$

	pH	OM	EC	C	N	C/N	IG
pH	-						
OM	-0.352	-					
EC	-0.017	0.110	-				
C	-0.352	1**	0.110	-			
N	-0.510	0.607*	0.613 *	0.607 *	-		
C/N	0.161	0.556	-0.405	0.556	-0.313	-	
IG	-0.006	-0.521	0.620 *	-0.521	0.394	-0.958 **	-
RES	0.16	-0.691*	0.600 *	-0.691	0.081	-0.855 **	0.940 **

3.4. Bacterial diversity by DGGE

The figure 1 illustrates the shift in the bacterial soil biomass composition, analyzed through DGGE, due to the different soil treatment. The DGGE band pattern is shown in Figure 1 and OMW spreading had an important effect on the development of the Bacteria domain.

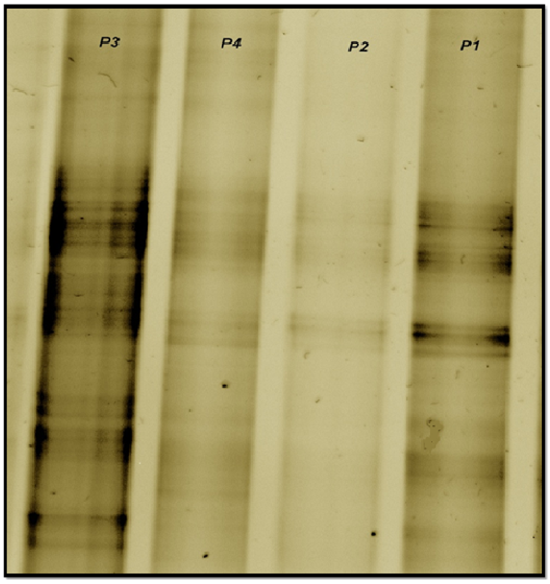

Figure 1. DGGE analysis of bacterial community in the different studied soils

The figure showed a higher number of the bands, in particular in P1, P3 and P4. In addition, the bands disposition remains very close, in spite the gradient gel concentration was repeated several times. In fact, the excised bands were impossible. However, this figure gives an idea onto the very important bacterial diversity in the different soils studied. P3 (Plot treated with OMW spreading) showed the highest number of bands and almost incomputable, with a very intense quality. Moreover, the effect of OMWs on the soil bacterial population diversity was shown in different works (Saadi *et al*., 2007; Di Serio *et al*., 2008; Barbera *et al*., 2013). In contrast, in the P2, (Plot with soil planted since 1900), an important number of bands completely disappeared. This result suggested the sol status in this plot traduced by the weak OM rate founded in this plot and reported in the physicochemical soil Properties Section. A later study can be applied to identify the different species detected in this work.

4. Conclusion

The techniques involved in this study for the fields Olive tree soil remediation in a dry semi zone showed an important impact. Indeed, an increase of the organic matter was revealed after the implication of theses processing. However, the effects of these processes seem to be contradictory for a technique closed view another one. If it made an increase of the soil fertility and biological activity after treatment with the introduction of the Fig tree in intercalary with Olive Tree. A pH reduction accompanied by a spectacular bacterial biodiversity was revealed by DGGE molecular technique in the plot treated with OMW spreading and a soil phytotoxicity in counterpart. A further perspective to conjugate the fig tree introduction technique to a moderate OMW spreading dose could be applied to enhance the physical and biological soil activity. Moreover, the statistical analysis of the soil characteristics showed a significant negative correlation between OM and Soil respiration, which can play an important rote to CO_2 sequestration.

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