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RESEARCH ARTICLE

Evolution of the microbial population of a cultivated soil with organic matter input under semi-arid conditions (Tiaret, Algeria)

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To establish the link between soil, plant and climate, it is necessary to study the soil biological state. The work is particularly concerned with monitoring the levels of microorganisms such as: actinomycetes, aerobic bacteria, azotobacters and fungi of a cultivated soil under exogenous organic matter input in the semi-arid region (Tiaret, Algeria). Amended soil samples were prepared from horse manure at different doses (0%, 20%, and 40%) and then put into a wheat crop. These samples were subjected to microbial characterization according to the different vegetative stages of wheat growth. The results obtained during this research revealed that the 20% horse manure doses that seem to be the most effective ($P<0.01$) and generated the stimulation of a good microbial proliferation and even more around the roots of the amended soils than in soil without a crop; coinciding with the tillering stage of the wheat crop ($P<0.001$). As could be predicted, the behavior of the microflora is related not only to the phenological stages of wheat growth but also to soil climatic conditions. Positive interactions have been demonstrated between plants, organic amendment and rhizosphere organisms with their heterogeneity.

Keywords: Algeria; Horse manure; Microbial population; Rhizosphere; Wheat; Vegetative stage

Introduction

Soil organic matter promotes a wide variety of habitats for microorganisms in soil (Bernoux & Chevallier, 2013); which, through its activities, produce humus without which a soil becomes dead, unsuitable for plants growth (Doucet, 2006), or degrades and then transforms it into a mineral form that can be assimilated by plants (Dridi and Toumi, 1999). For this reason, biologists believe that soil organic matter contributes importantly to soil fertility and productivity (Ananyeva et al., 2019) and that soil biological health is one of the best diagnostic indicators of soil quality (Ullah et al., 2014). So, scientists agree that the fact that growth of bacteria promoting can help the plant to function normally by removing the pathogen (Akhtar et al., 2018).

Naturally, soils in arid and semi-arid areas are poor in organic matter due to the low productivity of agro-ecosystems they support (Bernoux & Chevallier, 2013). Nutrients exported from crops in already poor soils are not adequately replaced (Mulaji, 2011). It is therefore urgent to develop short-term fertilization techniques that are more easily accessible to farmers. These techniques include organic fertilization through the use of manure, compost, green manure, nitrogen-fixing legumes (Nyembo et al. 2012, Gala et al. 2011); this context corresponds to our research problematic because the importance of organic matter inputs considerably modifies the proportions of soil/organic matter compared to what is usual in agriculture and can modify the evolution of organic matter. It is therefore essential to characterize this evolution (Grosbellet, 2008).

There are few data published on the evolution of soil microbial population related to land management practices such as exogenous organic matter addition. However, there are many studies on variations in the soil microbial density under the influence of: soil tillage (Bouchenafa et al., 2014), soil physico-chemical properties and altitude (Rebati et al., 2019) and soil irrigation (Lisetskii & Vladimirov, 2019). Moreover, in previous work on a similar region (Oulbachir, 1997) studied the evaluation of soil microflora in parallel with its dynamics, expressed by mineralizing power, in particular carbon mineralization. However, it is very clear that studies on this type of soil are still insufficient, particularly in North Africa and even more in Algeria where these types of soil occupy large areas. This is why we propose this work with the aim to bring more knowledge on the evolution of this type of soils in Algeria in an agricultural context.

We will try to assess the impact of organic amendments such as horse manure in order to measure the value of animal excrement on the evolution of biological indicators (quality) of our poor soils. This will allow better management of these inputs, the exogenous organic matter "(MOE, not produced on the plot), to improve and manage these soils in a bioeconomic way.

Materials and Methods

Study area

The study was carried at the experimental station of Sébaine (Technical Institute of Great Cultures), Dahmouni, wilaya of Tiaret which is part of the high cereal plains of western Algeria, whose coordinates are x = 1° 36" 27', y = 35° 27"32,4' and z = 960 m. The climate is semi-arid Mediterranean with a mean annual temperature is around 15, 4°C and mean annual rainfall of 410 mm (Figure 1).

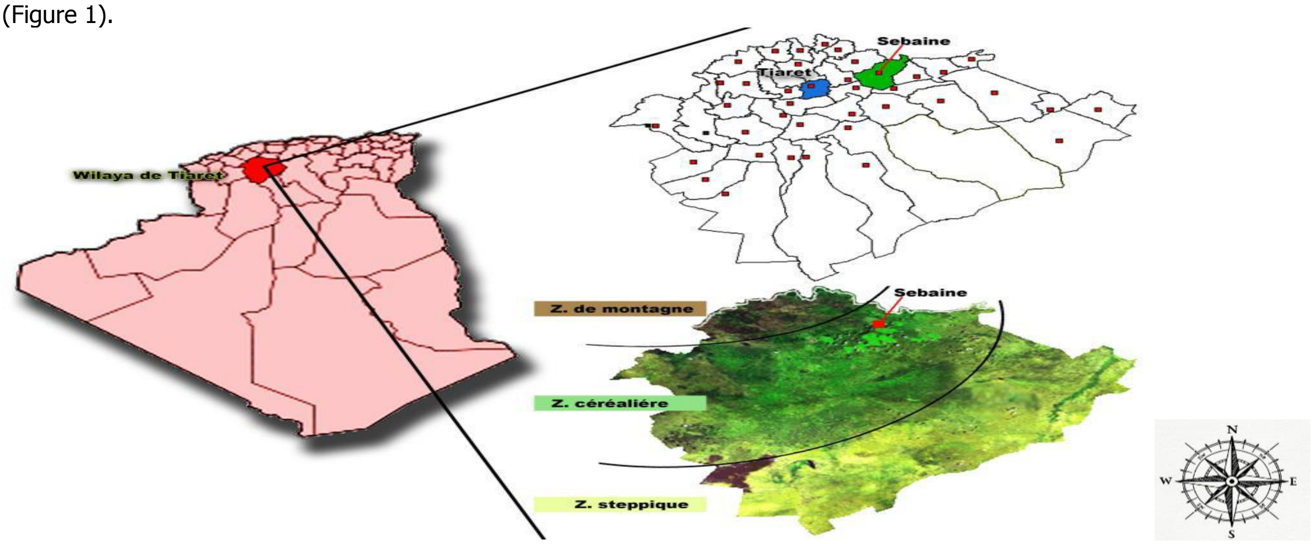


Figure 1. The map of the experimental station of Sébaine, Tiaret (Tahani, 2009).

Horse manure collection

Horse manure was collected from Horse Breeding Center, Tiaret (Algeria), it is fresh manure made up of a mixture of straw and excrement from horses of different breeds "Beards, Arab-Beard, Arabian Purcent and French Selle". After air drying, representative sample manure was taken and transferred to the laboratory (Table 1) of Agro-Biotechnology and Nutrition in Semi-Arid Zone's at the University of Tiaret, Algéria and analyzed for pH and electrical conductivity (Mathieu et al., 2003), total organic matter (Kebir, 2012), N (Oiu et al., 2010), P and K (Siboukeur, 2013).

Table 1. Physico-chemical characterization of horse manure.

Parameters	**pH (water)**	**EC (dS.m^{-1})**	**MO (%)**	**N (%)**	**P (%)**	**K (%)**
FC	8,15	10,38	40,34	1,2	0,36	0,93

FC: horse manure; pH: Potential Hydrogen; EC: Electrical Conductivity; MO: Organic Matter; N: Total Nitrogen; P: Phosphorus; K: Potassium.

Experimental site installation

Before experimentation (T0: November 12th, 2018), composite initial soil samples were taken from the experimental site (0-20 cm), were air dried, crushed and sieved to 2 mm and analyzed for its characterization and considered as a reference base for this study (Table 2).

Table 2. Characteristics of the control soil.

Parameters	**pH (water)**	**EC (dS m^{-1})**	**MO (%)**	**N (%)**
C	7,25	0,10	1,59	0,16

C: control soil; pH: Potential Hydrogen; EC: Electrical Conductivity; MO: Organic Matter; Total N: Total Nitrogen.

Fresh horse manure was well mixed and inverted into the top soil before sowing. Therefore, the experiment was laid out in the block-type split plot (23 m × 23 m) with three replications compares two treatments to a soil without manure (FC0); FC20: soil at 20% Horse Manure and FC40: soil at 40% Horse Manure hence the need to maintain a living vegetation cover on the surface of these soils.

Soil analyses

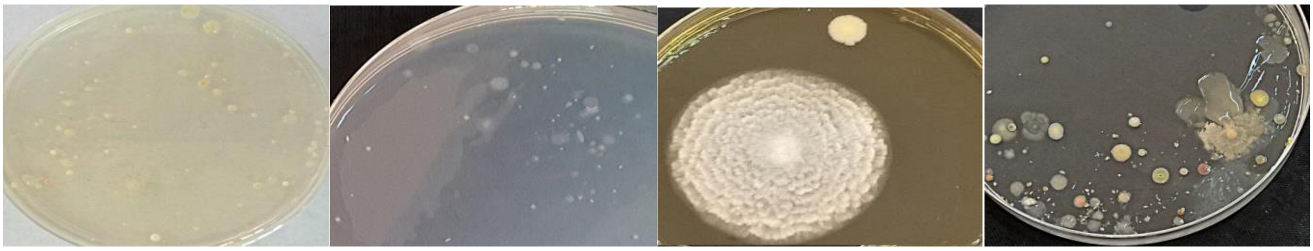
After wheat cultivation, the microorganisms namely: (aerobic bacteria, fungi, actinomycetes and azotobacters) and chemical properties were determined according to the different vegetative stages of wheat (sowing (T1), emergence (T2), tillering (T3) and upstream (T4)) corresponding to 21, 70 and 119 days, respectively, after the sowing wheat date (December 12th, 2018).

For soil chemical properties, the analytical methods used are: pH determined by the electrometric method (Mathieu et al., 2003) and organic matter through organic carbon determined by the ANNE method (1945). Moreover, for the estimation of the soil microbial population, we used the indirect method: MPN «Most Probable Number» method was used to assess the abundance of microorganisms and is very useful for "characterizing" soil samples (Saravanakumer et al., 2016). The principle of the method is based on: (i) solid cultures in which each group of microorganisms requires a specific medium favorable to their development (Table 3); (ii) seeding with suspensions-soil dilutions 10-5 or 10-7 (depending on soil type); (iii) reading the results after 7 days of incubation at 28°C by counting the number of colonies developed (Figure 2) per petri dish with the naked eye or with a magnifying glass (Oulbachir, 2014).

Table 3. Composition of culture media of different microorganisms.

Culture medium	**Aerobic bacteria**	**azotobacters**	**Fungi**	**Actinomycetes**
Composition and quantity by 1L distilled water + 20g agar	$C_{12}H_{22}O_{11}$ (10g) $C_5H_8NNaO_4$ (1,5g) K_2HPO_4 (0,5g) $MgSO_4$ (0,2g) $CaCO_3$ (0,2g)	Glucose (10g) K_2HPO_4 (0,2g) $MgSO_4$ (0,2g) K_2SO_4 (0,1g) $CaCO_3$ (5g)	$C_{12}H_{22}O_{11}$ (30g) K2HPO4 (1g) $MgSO_4$ (0,5g) $NaNO_3$ (3g) Kcl (0,5g)	$C_{12}H_{22}O_{11}$ (10g) $C_5H_8NNaO_4$ (10g) K_2HPO_4 (1g)

$C_{12}H_{22}O_{11}$: Sucrose, $C_6H_{12}0_6$: Glucose, $C_5H_8NNaO_4$: Sodium glutamate, K_2HPO_4: Dipotassium phosphate, $MgSO_4$: Magnésium sulfate, $CaCO_3$: Calcium carbonate, K_2SO_4: Potassium sulfate, $NaNO_3$: Sodium nitrate, KCl: Potassium chloride.



Legend:

A: Aerobic bacteria **B**: Azotobacters **C**: Fungi **D**: Actinomycetes

Figure 2. Macroscopic appearance colonies of different microorganisms (Benouadah, 2019).

Data Analysis

The data set was analyzed by one-way analysis of variance (ANOVA); it was used to study the effect of organic matter input on soil microbial density and chemical properties. This analysis is conducted by R Software.

Results

Microbial population of the studied soils according to the different vegetative stages of wheat growth.

This part is primarily intended for estimating the different groups of microorganisms and total microbial biomass according to the different vegetative stages of wheat growth, which makes it possible to assess the population fluctuations under the influence of various conditions, namely: the presence or lack of culture; human intervention, particularly in organic input, or, through their contribution to the carbon cycle and mineralization processes, as well as in the humification of organic matter.

The results obtained indicate that the control soil at time T0 included a defined microbial population composed of: 221, 66.10^6 aerobic bacteria, 111, 33.10^6 azotobacters, 257.10^6 fungi and 112, 66.10^6 actinomycetes per gram of soil.

Results also showed that the different microbial germs evolve differently, the examination of the results (Figure 3) indicates that the number of aerobic bacteria is significant at time T1, in particular in FC0 and FC20, which probably result from the development of organotrophic bacteria using organic substances. As a source of energy and nutritional group, there is a decrease in T2 time, which can be explained by exhaustion of this source and coincides with a period of low temperatures and, according to Oulbachir (2010) have a direct effect on the soil microflora.

At time T3; the number of aerobic bacteria rises (especially FC20 and FC40). This increase could be explained on the one hand by the intense physiological activity of the wheat, and on the other hand by the effect of organic stage where root exudation stimulates this group which continues to see a decrease in T4 where the wheat begins its maturation phase.

Moreover, we can see that the evolution of azotobacters takes the same rate as that of aerobic bacteria. They are important in T1 time, especially in soils 2 and 3 (FC20 and FC40), decrease at T2 time due to low temperatures, increase again at time T3 and then decrease in T4 (Figure 4).

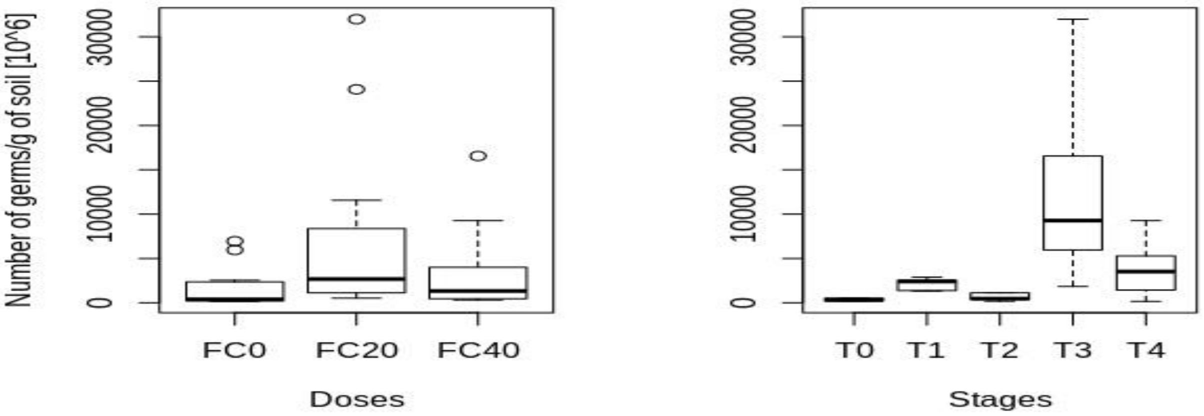


Figure 3. Evolution of aerobic bacteria.

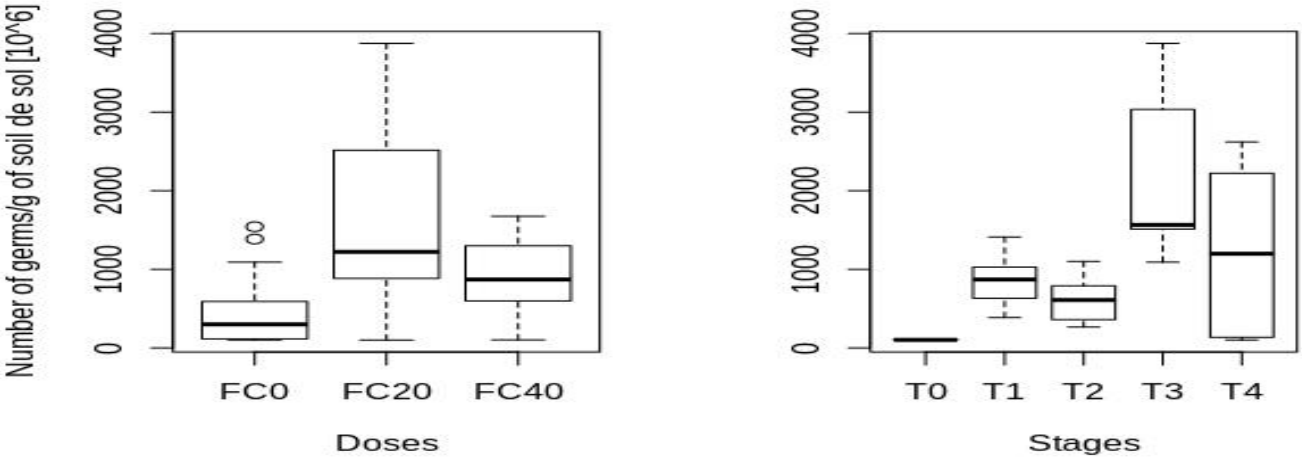


Figure 4. Evolution of azotobacters.

The results (Figures 5 & 6) showed that fungi and actinomycetes follow the same evolution during evolution where their density is relatively average at T1 and T2 times, then increase considerably at time T3, particularly in FC40 for fungi, and in FC20 for actinomycetes, then decrease in T4.

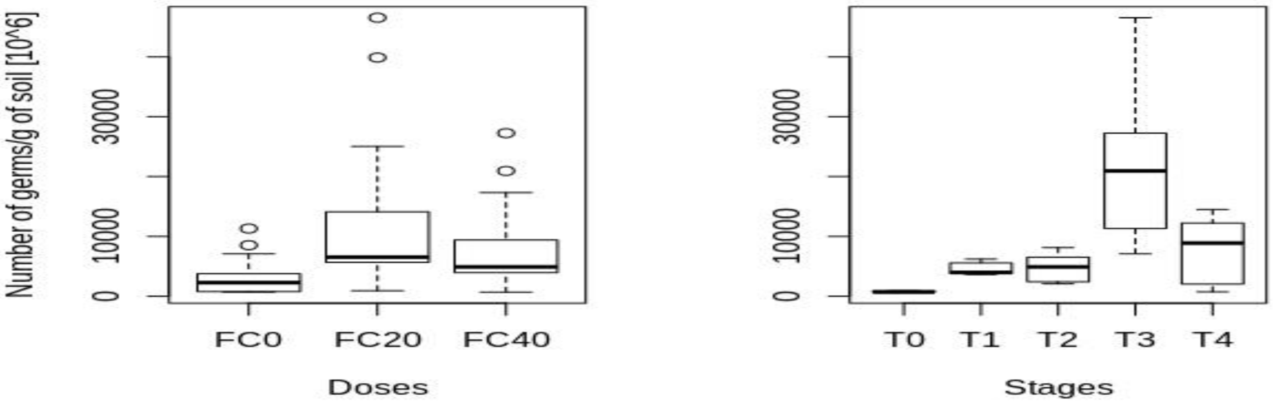


Figure 5. Evolution of fungi.

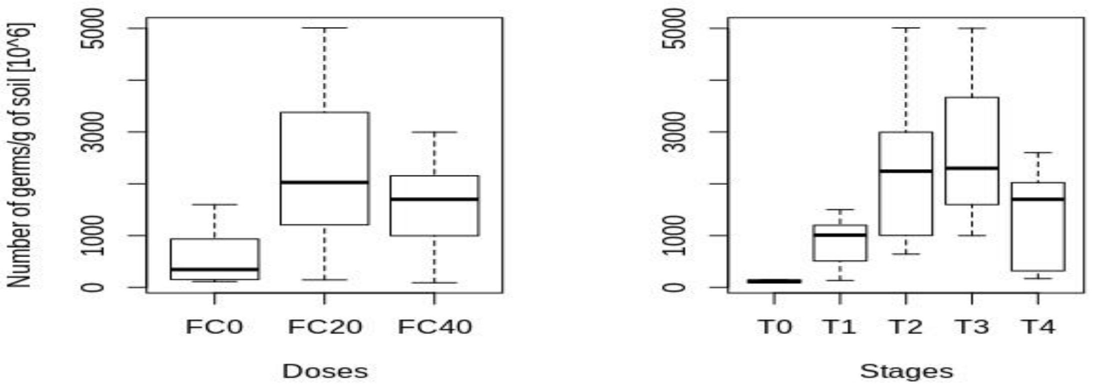


Figure 6. Evolution of actinomycetes.

To summarize the main observations and remarks, we present the evolution of the global microbial population of the soil, namely: actinomycetes, aerobic bacteria, azotobacters and fungi according to the different vegetative stages of wheat growth, which itself presents a stimulating parameter for microbial growth in Figure 7.

Initially, our control soil had a microbial density of (702,65. 10^6 germs/gram of soil). After organic supply and installation of wheat cultivation, a significant relative microbial biomass of (3723,947.10^6 germs/gram in FC0) is recorded at time T1, (5850,207.10^6 germs/gram in FC20) and (3981,933.10^6 germs/gram in FC40) then a decrease of these in T2 corresponding to (2260,07.10^6germs/gram FC0); and a slight rise in particular for amended soils (7065, 95.10^6 germs/gram in FC20) and (5016,92.10^6germs/gram in FC40).

At time T3: it is clear that the increase in microbial density is maximal (37147,02. 10^6germs/gram in FC20) coinciding with the tillering stage. Then, the microbial density decreased to: (1204,8. 10^6 germs.g^{-1} FC0), 13475,01.10^6 germs.g^{-1} FC20) and 8947, 653.10^6 germs.g^{-1} in FC40) where it was found that at time T4, this causes a decrease in root activity; which over time will become root litter. ANOVA analysis (Tables 4 and 5) also demonstraded a significant effect of soil organic matter'dose ($P<0,05$) and a very highly significant effect of wheat growth stage ($P<0,001$) on microbial communities. It was observed that microbial population were significantly affected by 20% horse manure dose (FC20) marked after the fourth sampling coinciding at the tillering stage.

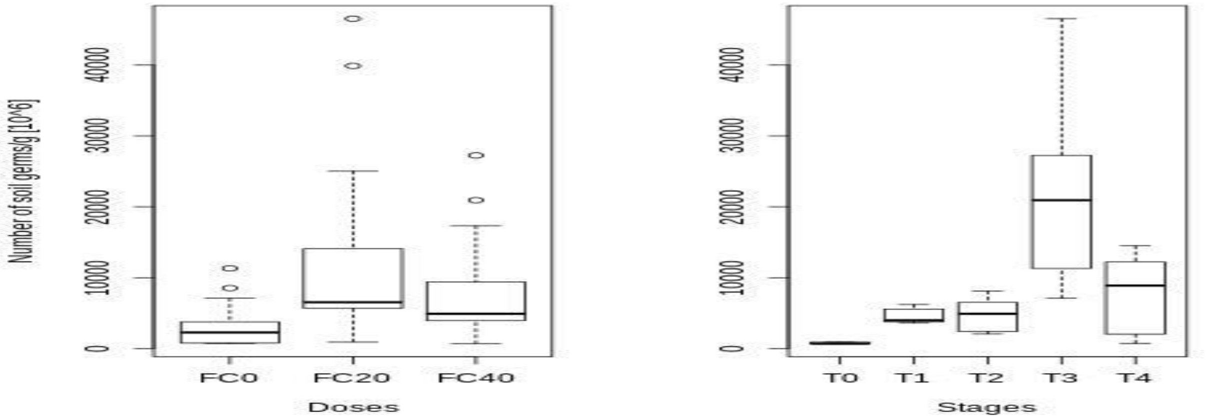


Figure 7. Evolution of the global soil microbial population.

Chemical characteristics of soil/horse manure mixtures according to the different vegetative stages of wheat growth.

Organic amendment also improved the soil chemical properties such as: soil organic matter and soil pH.

Soil total organic matter contents affected by organic amendment addition are summarized in Figure 8. The results obtained indicate that the organic amendment had a significant effect on total soil organic carbon (SOC) content in comparison to the control soil, with values 1,59%, 2,03% and 2,80% for FC0, FC20 and FC40, respectively, were recorded after spreading manure in the top soil. In the first and second vegetative stages of wheat growth, slightly decrease on total soil organic carbon (SOC) contents were observed due to the development of heterotrophic microorganisms that use organic matter as a source of energy and nutrition and then an increase was recorded at time T3 when living roots provide their environment with energy substances in significant amounts.

The data for the fourth growth stage total SOM was further improved highly significant ($P<0.01$) with higher that maximum values were recorded in FC40 (4,27%) and FC20 (2,98%) followed by FC0 (2,28%) due to the transformation of dead roots into root litter (Oulbachir, 2010). ANOVA analysis (Tables 4 and 5) also proved a very highly significant effect of doses organic matter input ($P<0,001$) and the stage of wheat growth (($P<0,001$) on soil' organic matter.

Moreover, the organic amendment significantly reduced the pH amended soils (Figure 9), this decrease could be explained by the decomposition of the organic matter that maintains a certain acidity in the soil, this is due in particular to the release of the organic acids and to the acidifying action of CO_2 released by the microbial activity. This acidity is partly responsible for the dissolution of poorly assimilable elements (Phosphate, Iron, etc.) in calcareous soils (Oustani, 2006). According to ANOVA variance analysis, there is a very highly significant difference (($P<0.001$) between the organic amendment dose and the soil pH. Moreover, there is not significant difference ($P>0.05$) between soil pH and the stage of wheat growth.

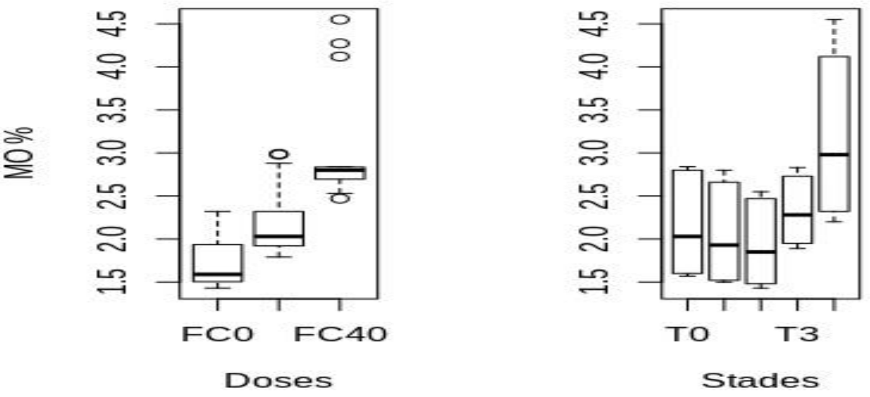


Figure 8. Evolution of soil organic matter.

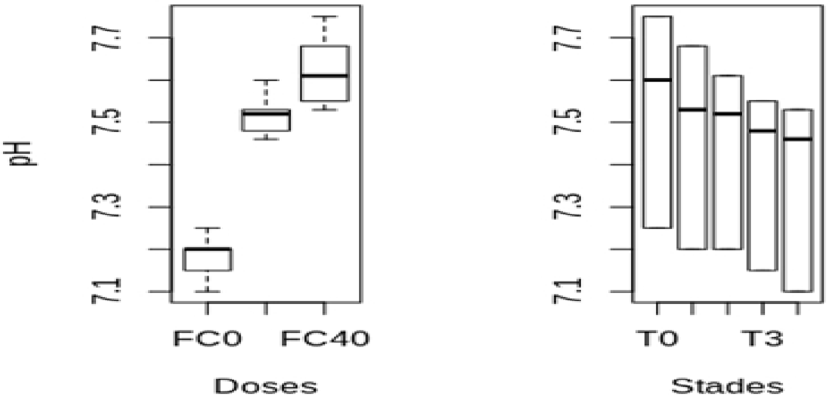


Figure 9. Evolution of soil pH.

Table 4. Output of the ANOVA analysis demonstrating the organic matter'dose on soil microbial density and soil chemical properties.

Soil property	Df	Sum Sq.	Mean Sq.	F	P
Soil microbial density	2	6,795e+08	339762090	3,854	0,0291*
% MO	2	12,83	6,413	26,24	4,04e-08 ***
pH	2	1,6131	0,8065	19	2e-16 ***

Significant codes: 0 '***' 0,001 '**' 0,01 '*' 0,05 '.' 0,1 ' ' 1

Table 5. Output of the ANOVA analysis demonstrating the stages of wheat growth on soil microbial density and soil chemical properties.

Soil property	Df	Sum Sq.	Mean Sq.	F	P
Soil microbial density	2	2.605e+09	651280023	14.66	1,86e-07***
%MO	4	8,829	2,2072	6,19	0,000565 ***
		0,1591	0,03977	0,977	0,431

Discussion

Our experimentation study aimed to determine the evolution of microbial population with organic matter input of a cultivated soil according to the different vegetative stages of wheat growth under semi-arid conditions in the region of Tiaret, Algeria. Our findings revealed that microbial communities were represented by the high concentration around the roots and even more in amended soil than in soil without culture. According to Lynch (1982), the presence of wheat in soil has a stimulatory effect on microbial biomass due to exudation, where root production provides readily available compounds, constituted by a readily biodegradable labile material that are responsible for stimulating microbial density, this stimulation is particularly expressed in the tillering stage.

However, it has been found that the presence of living roots in wheat cultivation, leading to exudation and root rhizodeposition, is at the origin of the microbial stimulation and its activity, which is manifested by an increase in biodegradation and mineralization (Oulbachir, 2010). In general, we notice that the microbial groups studied evolve differently depending on the conditions to which they are linked to so it'is necessary to show the extreme heterogeneity of the distribution of the microbial population in the rhizosphere. According to Oulbachir (2014), it is due to a temporal variation where the root meets a diversity of microorganisms in its growth. The success of colonization depends on the mechanisms of adhesion of the bacterium to the root wall, affinity of the substrate and probably recognition substrate of plant or microbial origin. To the heterogeneity due to the variation of the substrate in time and space, it is necessary to add the heterogeneity of the root environment (Golemen, 1985). The results obtained indicate that there is a certain parallelism between the climatic rhythm and the vegetative rhythm of the crop. It is obvious that at the tillering stage, microbial germs reach its relative maximum in the different soils; at a time when the living roots provide their environment with energy substances. It has appeared in work done (Oulbachir, 2010). These energy substances are easily assimilated, stimulating rhizospheric microflora. These observations are also consistent with those of Vilain (1987), who also obtained a particularly high density at tillering but very low after maturity when the root exudation is reduced.

Cultural practices, such as manure, improve plant growth, resulting in an increase in rhizodeposition, a carbon source that is easily metabolized by microorganisms in the rhizospheric. Therefore, cultural practices which have an indirect effect on microbial population, but the application of these organic amendment either at appropriate doses because the decomposition of large quantities of organic matter accumulated on the soil surface can lead to temporary anaerobiosis: these are arguments in favor of a biochemical toxicity rather than parasitic, especially if high humidity promotes microbial growth while oxygen diffusion is slower. Under these conditions, phytotoxic organic acids can be formed (Koull and Halilat, 2016, Davet, 1996).

The results obtained indicated that application of organic amendments improved significantly the soil organic matter and the soil pH. Therefore, Samreen (2017) also indicate that organic amendment such as farm yard manure had a significant effect on total soil organic carbon (SOC) content. However, the variation of these parameters considerably changes the density of the microbial population of the soil (Koull and Halilat, 2016) which, according to Bernoux & Chevallier (2013); soil organic matter (MOS) is essential for the biological activity: it is the main source of energy and nutrients for soil organisms. Moreover, the MOS favors a wide variety of habitats for the microflora (fungi, algae, microorganisms, etc.) in the soil. Most species are found in the first 2-3 cm of soil where the concentrations of organic matter and roots are highest. For this reason, soil organic matter content (SOM) is an important index to measure the level of soil function and soil quality, and detection of SOM content is an important approach to understand the local soil fertility (Jingzhe et al., 2017).

Moreover, Wang et al. (2014), Lauber et al. (2008) found that soil microbial composition and activity of the microorganisms were strongly influenced by the soil pH and each microbial species is active between pH limits, with an optimal value (Dari, 2013): fungi are generally predominant in acidic soils; capable of thriving over a wide pH range, averaging between 3,5 and 8,5 while bacteria predominate in neutral or slightly alkaline soils, such that actinomycetes prefer pH 6 to 7,5, which means microbial ecology of germs (Bouchenafa et al., 2014). However, Frey et al. (1999) found contradictory results compared to our measurements, in particular that the soil organic matter and the soil pH don't influence the fungal biomass.

Conclusion

Positive interactions between plants and micro-organisms have been demonstrated by heterogeneous rhizospheric organisms where the results obtained during this research have revealed a more intense microbial density and even more around the roots than in soil without culture, it is the substrates provided by root exudation and rhizodeposition that are responsible for stimulating microbial density. It should therefore be kept mind that the optimal rhisospheric effect corresponding to the vegetative growth period coincides in our case with the tillering stage. However, at the maturity stage, the rhizospheric effect decreases, reflecting a microbial degradation due to the litter effect of the dead roots. As could be predicted, the behavior of the microflora is related not only to the phenological stages of wheat growth, but also to the soil climatic conditions. We also observed, the effect of the organic amendment on the biological aspect of the soil or crop succession in some cases, fed the soil with organic matter (nutritional and energy source for microorganisms). In others, it is the 20% dose of horse manure that seems to be the most effective.

Acknowledgements

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