




## Article

# Impact of Management Practices on Soil Organic Carbon Content and Microbial Diversity Under Semi-Arid Conditions

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**Abstract:** Globally, arid and semi-arid agricultural land is characterized by low soil organic carbon (SOC) content. This impacts on the abundance and diversity of soil microorganisms in such environments. We therefore examined SOC and bacterial community structure dynamics in the single plots of the conventional (PC), improved fertilization (PA) and unimproved control (PT) at El Hmadna experimental station (Northwest Algeria) during five-time intervals T(0), T(15), T(70), T(104) and T(147 days). The SOC content was determined using the modified Walkley and Black method. The 16S rRNA genes were isolated from soils and sequenced using the Illumina sequencing platform. Over time, OC levels increased by more than 15%, especially in the improved plot. The highest OC stock was observed for the unmanaged control plot (47 Mg ha<sup>-1</sup>), also associated with higher bacterial biomass. However, taxonomic analysis revealed that bacterial diversity was higher in PA and PC, with Actinobacteria (42%) and Firmicutes (15%) dominating. Soil salinity did negatively influence SOC but the imposed management practices such as organic amendments did improve both carbon retention and bacterial diversity. The results underline the importance of imposing sustainable agricultural practices to improve carbon sequestration and soil health in semi-arid regions.

**Keywords:** semi-arid area; organic carbon dynamics; bacterial microflora; physicochemical properties; soil management practices; clay soil



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## 1. Introduction

Arid and semi-arid lands constitute approximately one-third of global terrestrial surface according to the United Nations Committee to Combat Desertification (UNCCD). These lands suffer from degraded and vulnerable soil, limited vegetation cover and low soil organic matter (SOM) with less than 4% [1]. Future projections for climate change in semi-arid areas indicate that global temperature will rise and the temporal and spatial patterns of precipitation will change [2]. Consequently, crop production and food security for one-third of the global population living in these areas will deteriorate [3]. Nevertheless,

arid and semi-arid soils' total potential of soil organic carbon (SOC) sequestration can reach up to 20 Pg C over 50 years [4]. Numerous studies indicate that this sequestration can be achieved through adopting land use and management practices emphasizing enhancing land vegetation cover and increasing SOM contents [5–7]. These practices not only aim to mitigate the effects of climate change but also to restore degraded soils and strengthen the resilience of agroecosystems in these vulnerable regions [4,7].

In addition to their role in ameliorating the physicochemical properties of the soil [8,9], SOM is a key factor limiting microbial growth and activity in the soil [10]. The interaction between SOM and soil microbiology enormously impacts the ecosystem's functionality [11]. A decline in SOM content results in a decrease in soil microbial biomass, a crucial indicator of soil health and thus a loss in the essential ecosystem functions and disturbance of the vital processes provided by soil [12].

In semi-arid environments, microbial biomass plays a critical role in nitrogen fixation [13,14] and soil carbon cycling [15]. The main function of microbial biomass is to decompose SOM to release the essential nutrients from their organic sources [16,17]. On the other hand, the microbial-derived residues produced upon their decomposition such as amino sugars, proteins, lipids, biopolymers and other molecules are considered a vital source of OC and N in the soil [18,19].

Typically, semi-arid soils display reduced microbial biomass and diversity. Among the dominant microbial communities detected in arid and semi-arid lands are Proteobacteria, Actinobacteria, Planctomycetota and Acidobacteriota [20,21]. The accumulation and diversity of microbial community structure in these environments are affected by lack of moisture, extreme variations in precipitation, high temperature and soil salinity [22,23]. However, these stresses can be influenced by environmental parameters such as climate, land use type and soil management practices [24–26]. Moreover, shifts in microbial communities under different management practices can provide important insights into the capacity of these soils to recover their biological functions and enhance carbon sequestration [11,25].

Adopting management practices such as fertilization or changing land use have significant and long-lasting effects on microbial activity in semi-arid soils [5,27,28]. Mineral and organic fertilization modifies C and N sources for microbial communities through a substantially higher input of organic matter below and above ground [6,29]. In a meta-analysis study, Allison and Martiny (2008) [30] found that 84% of 38 studies reported that microbial community composition is sensitive to N, P and K fertilization. Wardle (1992) [31] indicated that C and N are limiting elements for soil microorganism accumulation in the soil. In another meta-analysis based on data from long-term fertilization trials in cropping systems, Geisseler and Sow (2014) [32] found that mineral fertilizer application results in a 15.1% increase in microbial biomass above levels in unfertilized control treatments. Sui (2019) [33] found that the beta diversity of the microbial community in semi-arid lands can be significantly affected by soil pH, available P, N and OC due to changes in land use. In their study, they indicated that arable land consistently showed higher alpha diversity for bacteria, Acidobacteria and fungi compared to other land use types. Plant species and biomass directly impact the availability of carbon and other nutrients in the soils, thus affecting microbial biomass's diversity, distribution and community composition [34].

Additionally, organic agricultural practices can significantly influence soil bacterial and fungal biomass; it was found that their diversity increases under no-tillage practices [35]. The activity of the microbial biomass is high in undisturbed soils compared to tilled soils as repeated tillage results in loss of SOM through rapid decomposition from improved aeration and exposure of protected OM to microbial mineralization [36,37]. Furthermore, undisturbed soil or fallow land management favors soil carbon sequestration and the microbial community structure [38]. Despite this knowledge, integrating microbial

community assessments with SOC dynamics under various land use intensities and soil amendments in semi-arid agriculture remains underexplored and represents a research gap that this study aims to address.

Studies concerning microbial communities and diversity responses to the OC dynamics in semi-arid lands are scarce. In Algeria, few studies addressed the relationship between bacterial microflora and OC accumulation and dynamics, particularly on clayey and saline agricultural soils. Previous studies focused on characterizing soil microbial diversity in hypersaline natural ecosystems, other studies explored the relationships between microbial biomass and OM accumulation [39–42]. Consequently, there is a lack of precise data on microbial density and diversity of agricultural soils, their relationship with OC dynamics and the impact of management practices on bacterial structure and diversity.

We therefore used genomic DNA analysis to examine changes in soil bacteria communities and their relationships with soil physiochemical properties in three plots receiving different management practices as follows: 1. a fallow or undisturbed land, used in this study as a control plot; 2. a conventional plot that receives regular mineral fertilization NPK; and 3. an amended plot that received NPK, sand, cattle manure, gypsum and ferrous sulfate amendments over a 3-year project.

Thus, our specific objectives of this study are: (i) to assess the effect of conventional and improved management practices on OC accumulation and dynamics in semi-arid soil; (ii) to elucidate the changes in soil bacterial structure and diversity affected by SOC and soil properties; and (iii) to investigate the role of bacterial communities in SOC storage.

The outcomes of this study will help quantify the influence of land management on soil bacterial community structure and diversity in northwestern Algeria. The research is of significant value for improving soil management practices in Algeria. Additionally, the findings are expected to provide actionable insights for sustainable soil restoration strategies and contribute to global knowledge on the interplay between microbial ecology and carbon dynamics in dryland agriculture.

## 2. Materials and Methods

### 2.1. Description of the Study Area

The experimental site is in the National Institute of Agronomic Research of Algeria (INRAA) experimental station in El Hmadna, region of Relizane (Northwest Algeria). The site coordinates are 35°55'26" N and 0°44'57" E at an altitude of 48 m (Figure 1). The station, which is surrounded by two mountain ranges, the Dahra to the north and the Ouarsenis to the south, covers an area of 77 ha and is part of Bas-Cheliff zone, which covers 2750 km<sup>2</sup> [43]. The climate is Mediterranean, characterized by hot summers, cold winters and poorly distributed annual rainfall. This area is also known as the “oven of the Tell” due to the extreme heat in the summer season [44]. The soil of the study area is a clay texture with high salinity [45].

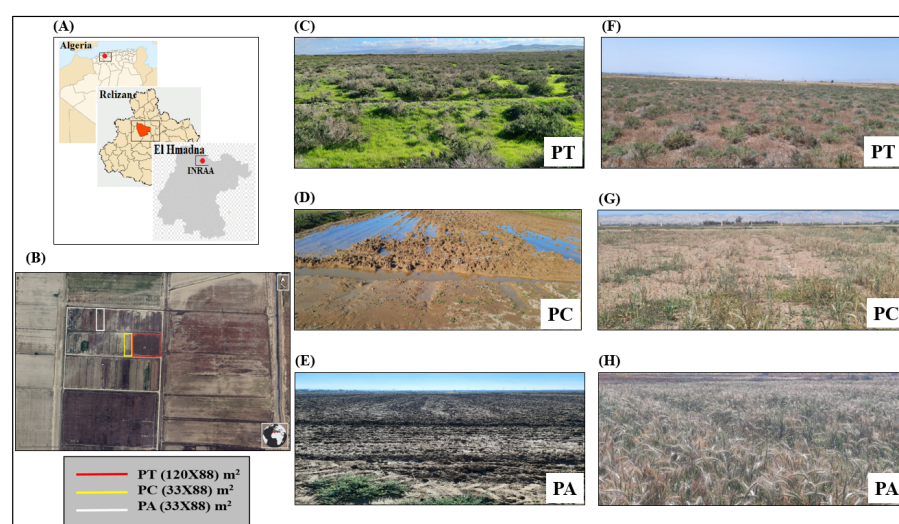
### 2.2. Description of the Plots

The study was conducted on three plots, as illustrated in Figure 1. Table 1 summarizes the experimental design, highlighting the management history, applied treatments and the main baseline soil physical and chemical characteristics of each plot. These parameters represent the stable properties that did not vary during the observation period.

### 2.3. Sampling Strategy

Five soil samples (0–30 cm depth) were collected from each plot to form a composite sample. Physicochemical analyses included OC content, texture, bulk density, salinity, pH, cation exchange capacity (CEC), total and active limestone, total nitrogen, available

phosphorus and potassium. Soil monitoring covered five time intervals: T(0), T(15), T(70), T(104) and T(147). Bacterial microflora was analyzed at two key stages: T(0), representing the initial state, and T(104), corresponding to the peak of microbial activity [46]. For each plot, a composite soil sample was prepared by homogenizing five subsamples collected randomly to ensure representativeness. The composite samples were then stored at 4 °C in sterile containers prior to analysis. The study covered both wet (November–April) and dry (May–October) seasons. Table 2 provides an overview of the sampling timeline, detailing the chronological progression and the specific objectives assigned to each sampling point.



**Figure 1.** (A,B) Geographical location of the experimental plots. (C–E) Plots in the wet period (December 2021). (F–H) Plots in the dry period before harvest (May 2022).

**Table 1.** Main characteristics of the experimental plots.

Plot	Treatments Applied During the Study	Prior Management (Before 2021)	Crop 2021–2022	Vegetation cover	Particle Size				Bulk Density (g/cm³)	CaCO <sub>3</sub> (%)
					Clay (%)	Fine Silt (%)	Coarse Silt (%)	Sand (%)		
PA	Plowing + NPK (15-15-15) 100 kg/plot	Algerian-Chinese project (3 years): NPK + cattle manure + sand + gypsum + ferrous sulfate	Rainfed soft wheat ( <i>Triticum aestivum</i> L.)	–	41	28	4	27	1.3	22
PC	Plowing + NPK (15-15-15) 100 kg/plot	NPK (15-15-15) 100 kg/plot	Rainfed soft wheat ( <i>Triticum aestivum</i> L.)	–	63	28	5	4	1.4	20
PT	None (uncultivated fallow since 1942)	No agronomic intervention	None	<i>Suaeda fruticosa</i>	61	30	2	7	1.4	21

PT: control plot, PC: conventional plot, PA: amended plot.

**Table 2.** Soil sampling schedule, growth stages, seasons and sampling purposes during the experimental period.

Stage (Days)	Date	Growth Stage	Season	Sampling Purpose
T(0)	14 September 2021	Before sowing	Dry	Physicochemical + bacterial analysis
T(15)	29 December 2021	Germination/emergence	Wet	Physicochemical analysis
T(70)	23 February 2022	Tillering	Wet	Physicochemical analysis
T(104)	29 March 2022	Bolting/heading	Wet	Physicochemical + bacterial analysis
T(147)	11 May 2022	Maturation	Dry	Physicochemical analysis

## 2.4. Physicochemical Analysis

Mineral fractions were measured using the “Robinson” method [47]. The principle of this method is to remove all cement-like carbonates, oxides and organic substances by oxidation with hydrogen peroxide. After dispersion in sodium hexametaphosphate, the samples were pipetted at different times and depths, following different sedimentation intervals. Sampling time and depth were calculated using Stokes’ law. All particle size data were expressed as a percentage of fine earth (<2 mm). pH was measured using a pH meter (Hanna Instruments, Netherlands) for suspensions with a 1:2.5 (*m/v*) ratio of fine earth and water. Salinity was measured by electrical conductivity (EC) in millisiemens per centimeter ( $\text{mS cm}^{-1}$ ) using a conductivity meter (Hanna Instruments, Netherlands). A suspension of fine earth and water with a ratio of 1:5 (*m/v*) was set for this analysis [48]. The bulk density (BD) in  $\text{g cm}^{-3}$  was calculated by direct sampling using a metal cylinder with a height of 5 cm and a diameter of 5.5 cm [49]. Samples taken by the cylinder were dried and passed through a 2 mm diameter sieve to measure coarse particle content (expressed in %). OC content was determined by the modified Walkley and Black method, based on the oxidation of OC by potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) in sulfuric acid [50]. Total limestone ( $\text{CaCO}_3$ ) content was determined by a Bernard calcimeter. The  $\text{CO}_2$  released by the reaction was measured by a gas burette [51]. Active limestone was measured according to the method described by Drouineau (1942) [52]. The excess ammonium oxalate was determined by titration with a solution of potassium permanganate in a sulfuric solution. Total nitrogen was determined by the Kjeldahl method described by Bremner (1965) [53]. Phosphorus content was measured according to Olsen (1954) [54] using a UV/visible spectrometer. The potassium content was measured based on dilute ammonium acetate with a flame spectrophotometer using a method described by Nash (1971) [55]. CEC was determined using the sodium acetate method reported by Rhoades (1982) [56].

## 2.5. Organic Carbon Stock Calculation (OCS)

The soil OCS was calculated for 0 to 30 cm depths using the equation recommended by the FAO [57,58] as follows:

$$\text{OCS} \left( \text{Mg C ha}^{-1} \right) = 0.1 \times C \times \text{BD} \times T \times (1 - \text{CP})$$

where OCS is organic carbon stock ( $\text{Mg C ha}^{-1}$ ), *C* is carbon content ( $\text{g C kg}^{-1}$  soil), BD is bulk density ( $\text{g cm}^{-3}$ ), *T* is the thickness of the soil horizon (cm) and CP is the gravel content or coarse particles ( $\text{g g}^{-1}$  soil).

## 2.6. Biological Soil Analysis

### 2.6.1. DNA Extraction and Polymerase Chain Reaction (PCR) Amplification

The genomic DNA was extracted from 250 g of soil using a DNeasy® PowerSoil® Pro Soil Kit (sourced from QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s instructions. This kit is effective in removing PCR inhibitors as it employs the second generation of QIAGEN Inhibitor Removal Technology® (IRT). Cell lysis occurs through both mechanical and chemical methods. Total genomic DNA is captured onto a silica membrane in a centrifuge column format, then washed, eluted and is ready for NGS, PCR and other molecular analyses, following procedures as previously described [59,60].

PCR amplification of the targeted 16S rRNA gene regions was performed using specific primers combined with barcodes. PCR products of appropriate size were selected by 2% agarose gel electrophoresis. Equal quantities of PCR products from each sample were pooled, end-repaired, A-tailed and ligated with Illumina adapters, according to standard protocols [61].



To ensure data quality and reliability, quality control (QC) was implemented at every step of the process, as shown in Figure S1. After sequencing, raw data containing low-quality sequences (“dirty data”) were processed to generate clean data through merging and filtering [60,62]. OTUs were clustered using UPARSE [62]. The representative sequence of each OTU was assigned a taxonomic classification using the SILVA 138 database [63] and the abundance distribution was calculated accordingly. The detailed workflow is shown in Figure S2.

### 2.6.2. Sequencing of the 16S rRNA Gene

Libraries were sequenced on an Illumina paired-end platform to generate 250 bp paired-end raw reads. The process of DNA library preparation, including PCR product selection, end-repair, A-tailing, adapter ligation and purification, was carried out following standard Illumina protocols, as summarized in Figure S3, which illustrates the key steps of the library construction workflow. The amplicon was then sequenced on the Illumina paired-end platform to produce 250 bp raw paired-end reads (Raw PE), which were subsequently merged and pre-processed to obtain clean tags [59]. Chimeric sequences were detected and removed using UCHIME [64], resulting in efficient tags used for downstream analysis.

Chimera checking and removal steps were performed to minimize false diversity estimates [61]. Final taxonomic annotation was performed using the SILVA 138 database, ensuring reliable taxonomic resolution [63].

### 2.6.3. Statistical Analyses

Matched reads were assigned to samples based on their unique barcodes and truncated by cutting the barcode and primer sequences. Matched reads were merged using FLASH (Version 1.2.7), a high-speed and accurate analysis tool designed to merge matched reads [59]. End reads when at least some of the reads overlap with the read generated from the opposite end of the same DNA fragment, and splice sequences were called raw tags. Quality filtering on the raw tags was performed under specific filtering conditions to obtain high-quality clean tags according to the Qiime (Version 1.7.0) quality-controlled process [60]. The tags were compared with the reference SILVA138 database using the UCHIME algorithm to detect chimeric sequences [61]. The chimeric sequences were then removed [62]. After obtaining the Effective Tags, sequence analysis was performed by Uparse software (Version 7.0.1090) [63]. Sequences with a similarity  $\geq 97\%$  were assigned to the same OTUs. The representative sequence of each OTU was examined for further annotation. For each representative sequence (Version 1.7.0) Qiime [64] in the Mothur method was performed against the SSUrRNA database of the SILVA138 database [65] for species annotation at each taxonomic rank (threshold: 0.8~1) (kingdom, phylum, class, order, family, genus, species) [66]. MUSCLE (Version 3.8.31) was used to understand the phylogenetic relationship between all representative OTU sequences [67]. The software can compare multiple sequences quickly. OTU abundance information was normalized using a sequence number standard corresponding to the sample with the fewest sequences. Subsequent analyses of alpha diversity were performed based on this normalized output data. Alpha diversity is applied in biodiversity complexity analysis for a sample across six indices, including Observed-species, Chao1, Shannon, Simpson, ACE and Good-coverage. All these indices were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). Linear regression was carried out to estimate the temporal OC response of each plot over the experimental period. Statistical analyses were performed using Python program (version 3.12.0), using the Pandas, Numpy, Mtplotlib.pyplot, Sklearn.linear\_model

and Sklearn.metrics libraries. Statistical significance of coefficients was assessed using the  $p$ -value and the student  $t$ -test.

### 3. Results

#### 3.1. Temporal Changes in Soil Physicochemical Properties

The temporal variation of key soil physicochemical properties across the different plots and sampling stages is presented in Table S1. The pH is neutral to slightly alkaline (7.0–8.1) at all sampling stages. The EC varied considerably throughout the different sampling periods. Plots PT and PC vary from excessively salty to slightly salty, while PA ranges from very salty to slightly salty. CEC is high to very high in all plots, which is typical for clay-dominated soils where cation exchange between soil and plant is relatively limited. The C/N ratio varies remarkably throughout the different samples. At T(0), T(15) and T(147), all plots recorded low to very low C/N ratios, indicating rapid OM decomposition. On the other hand, at T(70) and T(104), C/N is very high, revealing reduced biological activity and slow OM decomposition. The phosphorus levels varied from high to very high for all samples; similarly, the potassium levels were high in the three plots.

#### 3.2. Organic Carbon Variations

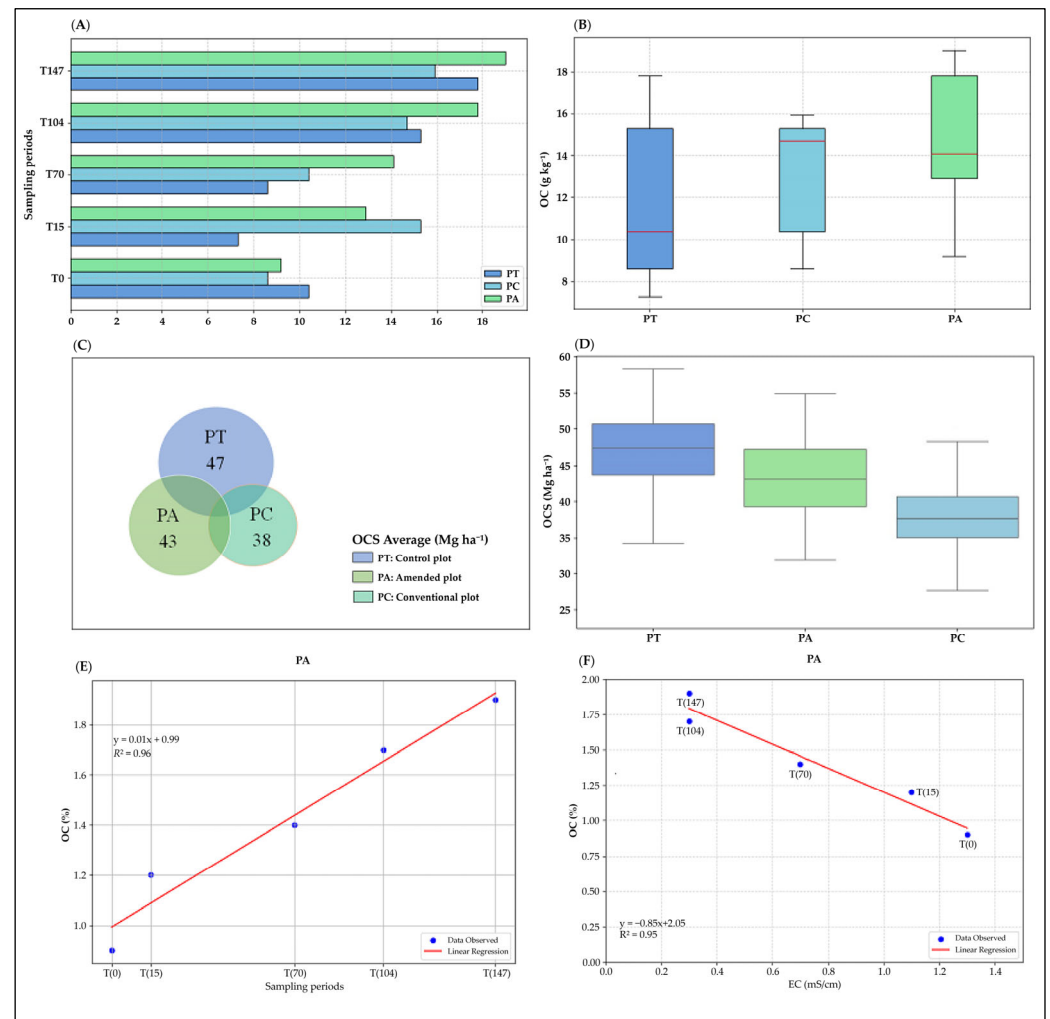
At T(0), OC content remained constant across all three plots; however, at T(15), it increased significantly in PC and PA, coinciding with plowing operations that incorporated fresh plant residues into the soil. This period also experienced 86.5 mm of cumulative rainfall and an average temperature of 19 °C; these conditions are favorable for OM mineralization. At T(70), OC content was higher in PA than in PC (Figure 2A), likely due to greater plant biomass, increased photosynthesis and enhanced rhizodeposition. Despite low rainfall (~5 mm), PA benefited from prior soil improvement practices that enhanced soil aeration and water retention, helping crops withstand the precipitation deficit. At T(104), a significant increase in OC levels was observed in all three plots (PA > PT > PC), while at T(147), OC levels rose further, particularly in PA. The last samples from PA and PC were taken before the harvest when the wheat crop was in its final vegetative phase, which may have increased carbon intake. Rainfall (~92 mm) during this stage may have also enhanced primary production, resulting in increased carbon inputs into the soil.

The dispersion of OC values is greater in the PA plot. The PT plot has the lowest OC concentration on average and the least dispersion (Figure 2B). Figure 2C,D indicate that PT displays the highest OCS values, with a median of 50 Mg ha<sup>-1</sup> and low data dispersion. PA shows intermediate values, with a median of 43 Mg ha<sup>-1</sup> and greater variability. PC has the lowest OCS, with a median of 40 Mg ha<sup>-1</sup> and a reduced total extent. PT seems to promote more OC storage than the other plots. PA also tends to favor greater OC accumulation than the PC plot.

A difference in plot means is noted, with PA > PC > PT. The variance reveals greater PC data homogeneity, while PA and PT show greater dispersion around the mean. The standard deviation indicates more stable and less dispersed data for PC, while PA shows the greatest variability. PT has a slightly higher standard deviation than PC and PT suggests moderate variability (Table S4). The  $p$ -values were well above the 0.05 threshold in all comparisons (PT vs. PC, PT vs. PA, PC vs. PA) (Table S5). This means that there is no statistically significant difference between the means of the PT, PC and PA plots.

Linear regression analysis reveals a strong positive relationship between sampling periods and OC values for PA (Figure 2E), with a very well-fitted model ( $R^2 = 0.96$ ) and a significant increase in OC of 0.0063 units per period ( $p = 0.003$ ). In contrast, for PT, although 69% of variability was explained ( $R^2 = 0.69$ ), the relationship was not significant ( $p = 0.08$ ), while for PC, the model was unreliable ( $R^2 = 0.30$ ,  $p = 0.3$ ), suggesting a limited influence

of time. Other factors could explain these variations, such as temperature, rainfall, soil texture, salinity, tillage intensity, fertilization and microbial activity.



**Figure 2.** (A) Variation of soil OC at each sampling stage. (B) Difference of soil OC variation among plots. (C) Variation in average of soil OCS. (D) Boxplots for difference of OCS variation. (E) Linear regression between sampling periods and OC values for PA. (F) Linear regression between EC and OC for PA. PT: control plot, PC: conventional plot, PA: amended plot.

Linear regression revealed a strong and statistically significant negative relationship between EC and OC for PA (Figure 2F), with 95% of OC variability explained by EC ( $R^2 = 0.95$ ,  $p = 0.005$ ). In contrast, PT showed a moderate but non-significant negative correlation ( $R^2 = 0.51$ ,  $p = 0.17$ ), while PC displayed a very weak and non-significant association ( $R^2 = 0.07$ ,  $p = 0.65$ ). These results highlight PA as the only site where EC strongly and reliably predicts OC changes, whereas the relationship is uncertain in PT and negligible in PC.

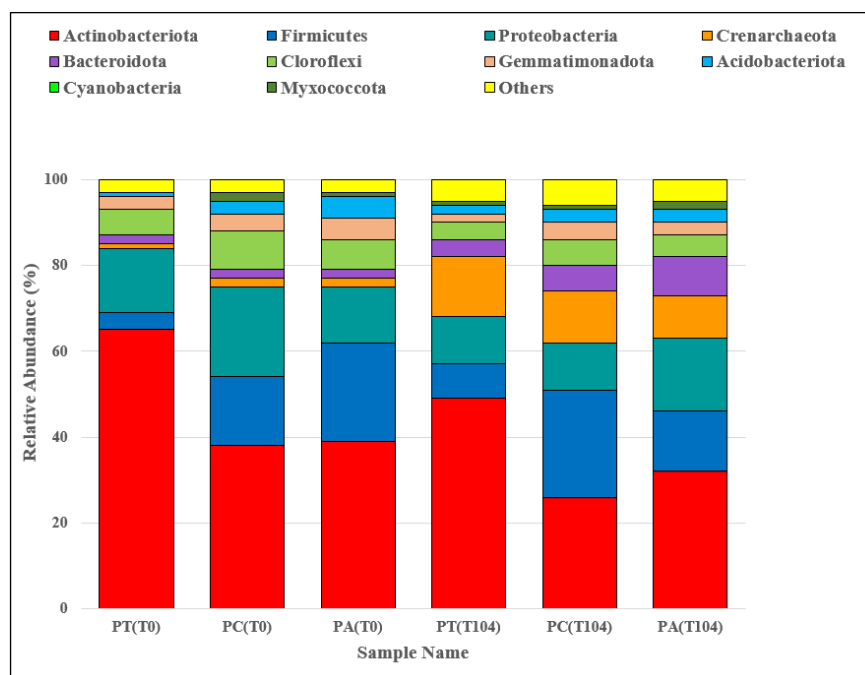
### 3.3. Composition and Structure of Bacterial Microflora

#### Identification of Operational Taxonomic Units (OTUs)

During the OTU construction process, basic information for each sample, including efficient tag data, low-frequency tag data and tag annotation data, was collected. The detailed summary of these steps is presented in Table S2, which shows the sequencing depth and data quality for all samples.



An average of 87,061 raw reads per sample was obtained. Following quality control procedures, an average of 77,981 valid reads per sample was retained for further analysis. The sequences were clustered into OTUs, resulting in a total of 2267 OTUs, which were taxonomically assigned using the SILVA138 database, as illustrated in Figure 3.



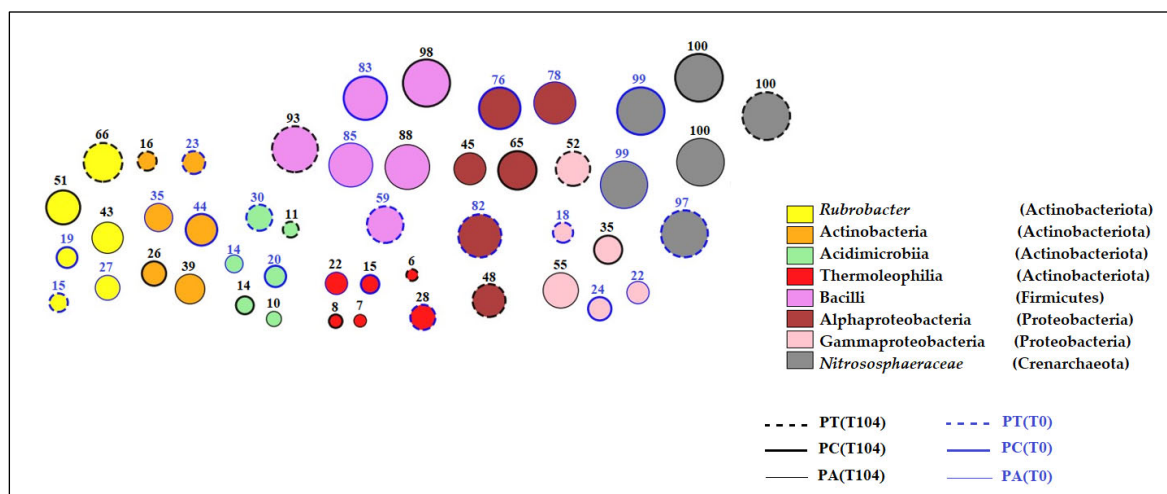
**Figure 3.** Taxa relative abundance in phylum. PT: control plot, PC: conventional plot, PA: amended plot.

Regarding the domain distribution, more than 98% of OTUs at T(0) were classified within the Bacteria domain, while only 2% were affiliated with the Archaea domain. At T(104), the proportion of Archaea increased to 12%, while the Bacteria domain represented 88% of the OTUs. The detailed summary of the tag counts and OTU numbers for each sample at both T(0) and T(104) is provided in Figure S4, which also highlights the distribution of sequencing effort and OTU richness across the PT (control), PC and PA plots.

Detailed analysis of the alpha diversity metrics presented in Table S3, including observed species, Shannon, Simpson, Chao1, ACE, Goods coverage and phylogenetic diversity (PD whole tree) indices reveals a close link between cropping practices and the structuring of microbial communities. As seen in Figure 3, the analysis showed the relative abundance of the different phyla. Complementing these results, the Krona display shown in Figure S5 provides a detailed visualization of the percentage contribution of each microbial group. It can be seen that at T(0), the dominant communities were Actinobacteriota (47%), followed by Firmicutes (14%) and Proteobacteria (16%), while the least represented included Cyanobacteria (<0.1%) and Myxococcota (1%). At T(104), the composition remained similar, with Actinobacteriota dominating (36%), followed by Firmicutes (16%) and Proteobacteria (13%), while Cyanobacteria (0.1%) and Myxococcota (1%) remained the least abundant. Overall, the relative abundances of the different microbial groups ranged from <0.1% to 47%, depending on the taxonomic group and sampling condition.

Figure 4 provides more details on the microbial structure of the four most abundant phyla. The OTUs with the highest indicator values for soils from the three plots at T(0) and T(104) were dominated by the phylum Actinobacteriota with the genus *Rubrobacter* and the classes Actinobacteria, Acidimicrobiia and Thermoleophila. Then came the Firmicutes with the class Bacilli, followed by the Proteobacteria, with the classes Alphaproteobacteria

and Gammaproteobacteria. Archaea are dominated by the Crenarchaeota, notably the *Nitrososphaeraceae* family.



**Figure 4.** Abundance of taxonomic ranks. PT: control plot, PC: conventional plot, PA: amended plot.

Analyses of beta diversity, as illustrated in Figure S6, reveal significant shifts in microbial community composition between T(0) and T(104). The UPGMA cluster tree based on Weighted UniFrac distance (Figure S6A) shows that the different treatments influenced not only the presence or absence of certain phyla but also their relative abundance, reflecting modifications in the fine structure and composition of bacterial communities across the plots.

In parallel, the UPGMA cluster tree based on Unweighted UniFrac distance (Figure S6B) emphasizes that the treatments affected the overall diversity of bacterial communities by altering both species richness and their phylogenetic structure. Together, these analyses underline the combined effects of management practices and seasonal variation on the microbial community assemblages at both taxonomic and phylogenetic levels.

## 4. Discussion

### 4.1. Organic Carbon Dynamics and Soil Physicochemical Properties

As detailed in Table S1, the OC levels increased notably at T(15) across the plots. This increase can be attributed to plowing, which enhances OM decomposition in the presence of optimal temperature and humidity, favoring carbon loss [68–70]. Additionally, the rise in OC may be linked to mineral fertilization amendments containing nitrogen, e.g., NPK or from atmospheric nitrogen fixed by N-fixing plants [71,72]. In addition, Table S1 shows a significant increase in total nitrogen content, especially in the PC plot, which likely supported enhanced OC storage through improved nitrogen availability [73]. Regarding salinity, the EC values reported in Table S1 highlight a marked reduction at T(104), particularly in the PA plot, likely due to heavy rainfall events during this period. This decrease in salinity was further supported by improved soil management practices in PA that facilitated better drainage and salt leaching, thereby contributing to the slowdown of OC mineralization [74]. At T(147), EC values from Table S1 confirm non-saline conditions in both PA and PC plots, which would further limit carbon mineralization.

Moreover, our linear regression analysis, supported by the EC and OC data from Table S1, revealed a clear negative correlation between salinity and OC levels, indicating that reduced salinity conditions are conducive to enhanced OC accumulation in these semi-arid soils.

#### 4.2. Organic Carbon Stock Variations

The fluctuations in OC averages have a direct impact on OCS. Higher concentrations and dispersion were observed in the PT plot (Figure 2C,D). The PT plot represents a fallow undisturbed natural ecosystem since 1942, therefore, C mineralization is negligible [75]. Previous studies indicated that soils with permanent vegetation cover and zero-till are characterized by a significant concentration of OM in the topsoil, higher microbial biomass (i.e., total tags for PT > 91400) and slower carbon mineralization [69,76]. Moreover, the high clay and carbonate contents might have influenced SOC resistance to mineralization through soil aggregate formation and stabilization [77–79]. The precipitation rate ( $\approx 53$  mm) at T(104) may have also contributed to the decrease in salinity and led to an increase in microbial activity and stimulating OM mineralization. Noteworthy is that the C/N ratio indicates low microbial activity at T(104) for all plots, which may have contributed to SOC storage.

#### 4.3. Variation in Soil Bacterial Biomass and Diversity

The results showed that bacterial biomass was lower during the wet period at T(104), with a total number of markers of 84,319 compared with 89,803 at T(0), despite a higher level of OCS at T(104) ( $53 \text{ Mg ha}^{-1}$ ) compared with T(0) ( $31 \text{ Mg ha}^{-1}$ ). On the other hand, bacterial diversity was higher during the wet period at T(104), with OTU giving indicator values of 2443 compared with 2091 for the dry period at T(0). The variation in microbial biomass and diversity is the result of several factors. In the wet period, elevated moisture and nutrient levels increase environmental and habitat diversity, creating more favorable conditions for microbial diversity. However, increased competition and fragmentation of by-product resources limit the dominance of certain species, thus reducing total biomass [80]. In dry periods, the more stressful conditions select a smaller number of resistant species, resulting in higher biomass but reduced diversity [81]. OC, although more abundant in wet periods, may be less accessible, while in dry periods, it is potentially more concentrated and labile, favoring higher bacterial biomass [82,83]. In the dry period at T(0), soil salinity in all three plots is high ( $1.5 \text{ mS cm}^{-1}$ ), while it decreases significantly in the wet period ( $0.3 \text{ mS cm}^{-1}$ ). The combined stress of salinity and drought reduces microbial diversity but favors the dominance of tolerant species, therefore maintaining high biomass [84,85].

The high density of the Actinobacteriota phylum in the PT plot may be attributed to the high vegetation cover [86]. Our results agree with previous studies showing that Actinobacteriota are of the dominant microbial communities in arid environments [87–89]. Unpredictably, the decline in OCS at T(0) despite the high density of Actinobacteriota may be explained by the high salinity levels at that time, which may have restricted the activity of the bacterial community [90]. Firmicutes dominated in the anthropized plots, i.e., PC and PA, and were mainly represented by the Bacilli class (Figures S5 and 4), which is probably due to soil management practices such as plowing and fertilization [91]. In contrast, the undisturbed PT plot showed a very low density of Firmicutes. Firmicutes can also resist unfavorable environmental conditions, such as high temperatures, drought and nutrient-poor soils [92]. In addition, the morphology and physiology of Firmicutes allow them to live in hypersaline and especially humus-bearing environments [42]. Among Firmicutes, the Bacilli class includes a significant concentration of halophilic species requiring very high OM rates. All three plots had high EC at T(0); however, at T(104) salinity decreased due to the high rainfall during this period, while OC content increased significantly, which may have amplified the density of the Bacilli class (Figures 4 and S5). Furthermore, the dominance of Firmicutes in the PC and PA plots at both T(0) and T(104) could be attributed to tillage practices affecting bacterial community composition. Firmicutes play a key role in soil fertility by contributing to the mineralization of OM and thus affecting the residence

time of the OC in the soil [93]. This was evident in the PC plot, which has the highest density of Firmicutes and demonstrates low OCS levels.

Proteobacteria are among the most abundant phyla after Actinobacteriota and Firmicutes. They are mainly represented by the classes Alphaproteobacteria and Gammaproteobacteria. Proteobacteria are nitrogen-fixing bacteria [94] and play a role in the carbon and sulfur cycles [94]. The Alphaproteobacteria class can adapt to dry, nutrient-poor soils, thrive in saline soils and is found in abundance in undisturbed and stable ecosystems [84]. Alphaproteobacteria were abundant in all three plots, particularly during the dry period at T(0) (Figure 4). In contrast, Gammaproteobacteria were dominant in the three plots during the wet period at T(104), when OC levels were higher compared with T(0). This phylum prefers moist soils rich in OM, tolerates saline soils and is more abundant in disturbed ecosystems [80,82].

Ammonia-oxidizing archaea are represented in our study by the Crenarchaeota class, dominated by the *Nitrososphaeraceae* family. The latter are abundant in warm, humid soils [95]. *Nitrososphaeraceae* are involved in the nitrification process [96]. They are most active in soils containing medium to high levels of OC (1–3%) [96]. Their activity is optimal in moderately humid environments with low to moderate salinity [97]. This explains why *Nitrososphaeraceae* are more abundant at T(104), which also explains the higher nitrogen levels at T(0). Biological nitrogen removal through nitrification could lead to OC loss [98], but this is not true for plots at T(104), where OCS is higher than at T(0). The hypothesis may still relate to soil salinity, which is very high at T(0) and may have significantly contributed to OC loss. High OCS values were observed at PT, particularly at T(104), where the reduction in salinity, probably due to rainfall (~53 mm), may have mitigated its limiting effect on microbial activity and OM mineralization. Despite unfavorable conditions at T(0), such as low OC content, high salinity and temperature, bacterial biomass was surprisingly higher; the results are in line with a previous study showing bacterial resilience in carbon-deficient soils [98]. The taxa were dominated by Actinobacteriota and Firmicutes, probably influenced by the clay texture and sand amendments. The observed higher bacterial biomass in PT at both T(0) and T(104) may likely be attributed to the dense root systems of permanent vegetation, which could enhance rhizosphere resource availability for microbial communities [99,100].

#### 4.4. The Role of Actinobacteria and Firmicutes in Soil Organic Carbon Dynamics

Our results reveal contrasting dynamics between Actinobacteria and Firmicutes in regulating SOC under semi-arid conditions, shaped by both environmental factors and management practices. The dominance of Actinobacteria (42%, mainly Rubrobacter) in the undisturbed control plot (PT) was associated with higher SOC stocks (47 Mg ha<sup>-1</sup>), suggesting a key role for this group in the long-term stabilization of soil OM. This observation aligns with the well-documented capacity of Actinobacteria to degrade complex polymers such as cellulose and lignin through the production of extracellular enzymes like cellulases and peroxidases [18]. Moreover, their resilience under dry conditions (T0), despite high salinity levels (EC = 1.8 mS/cm), can be attributed to their robust cell walls and production of hydrophobic metabolites [101]. Conversely, Firmicutes (15%, predominantly Bacilli) were more abundant in the managed plots (PA, PC), where their presence was negatively correlated with SOC stocks (40–43 Mg ha<sup>-1</sup>). This supports the hypothesis that Firmicutes may contribute to the accelerated turnover of labile carbon via fermentative pathways [81]. Their increased abundance under the influence of organic amendments and tillage reflects their sensitivity to anthropogenic disturbances [27].

Although the statistical analysis did not reveal significant differences ( $p > 0.05$ ) in SOC between plots, a 15% increase was observed in the PA plot. This apparent discrepancy can

be attributed to (1) the relatively short duration of the experiment, which may be insufficient for SOC changes to reach statistically detectable levels and (2) the inherently high spatial variability in semi-arid soils, which often limits the statistical power of short-term studies. From a biological perspective, even a non-significant 15% increase in SOC could have meaningful functional implications in these systems. Previous studies have demonstrated that even modest increases in SOC (5–15%) can enhance aggregate stability [102], stimulate microbial activity [103] and improve water retention [104]. These effects are particularly relevant in soils with low organic matter content, where minor improvements can positively impact crop productivity [103,104]. Therefore, while these findings remain preliminary, they highlight the potential of organic amendments to progressively improve soil quality in semi-arid areas. Long-term monitoring is, however, needed to confirm the sustainability of these trends.

Seasonal dynamics further revealed that during the wet period (T104), microbial diversity increased alongside a marked emergence of nitrifying Archaea (Nitrososphaeraceae), while overall microbial biomass appeared to decrease, likely due to intensified competition [105]. In contrast, during the dry period (T0), the community structure was dominated by stress-tolerant taxa such as Actinobacteria, leading to reduced SOC mineralization. Importantly, the strong negative correlation observed between EC and SOC in the PA plot ( $R^2 = 0.95$ ,  $p = 0.005$ ) highlights the inhibitory effect of salinity on microbial-driven carbon sequestration processes, consistent with the findings of Rath and Rousk (2015) [84] on the suppressive effects of salts on enzymatic activities.

Overall, these findings suggest that undisturbed systems (PT) maintain microbial communities that favor SOC retention over the long term; organic amendment practices (PA), although subject to variability, may contribute to mitigating SOC losses by supporting higher microbial diversity; salinity emerges as a critical limiting factor, acting independently of soil moisture regimes and potentially constraining microbial activity and SOC dynamics.

## 5. Conclusions

This study evaluated how soil management practices influence SOC dynamics and microbial diversity under semi-arid conditions in Northwest Algeria. Land management practices significantly influenced bacterial diversity, biomass and SOC dynamics in Algeria's semi-arid soils. During dry periods in the experiment, the PC plot showed high bacterial diversity (due to clay content) but lower biomass from salinity stress, while the undisturbed PT plot maintained the highest biomass. During wet periods, amended PA plots surpassed others in diversity due to improved soil conditions, whereas PC plots showed stress-adapted biomass increases. Undisturbed PT plots demonstrated resilient microbial communities and superior long-term SOC storage, while amended PA soils enhanced microbial diversity and soil properties. Conventional PC practices reduced SOC and favored stress-resistant bacteria. These findings underscore that sustainable practices (organic amendments, reduced tillage) optimize carbon sequestration, microbial health and ecosystem resilience in semi-arid regions like Algeria and beyond. Additionally, this study highlights the need to integrate drought and salinity into SOC models, assess long-term impacts of organic amendments and explore Actinobacteria and Firmicutes interactions across climates variability.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/land14051126/s1>, Figure S1. The procedures from DNA extraction to sequencing, Figure S2. The procedures from DNA samples to final data, Figure S3. Workflow of library construction, Figure S4. Summary of the Tags and number of OTUs in each sample for T(0) and T(104) samples. PT: control plot, PC: conventional plot, PA: amended plot, Figure S5. KRONA display, Figure S6. UPGMA cluster tree: (A) based on Weighted Unifrac distance; (B) based on



Unweighted Unifrac distance, Table S1. Soil physicochemical characteristics of different plots at each sampling stage, Table S2. Quality control statistics (QC), Table S3. Alpha diversity indice, Table S4. Descriptive statistics of OC values for studied plots, Table S5. Student's t-Test: Comparing OC differences between plots.

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## Abbreviations

Abbreviation	Definition
16S rRNA	16S ribosomal RNA (gene used for bacterial studies)
BD	Bulk density
C/N	Carbon-to nitrogen ratio
CaCO <sub>3</sub>	Calcium carbonate
CEC	Cation exchange capacity
DNA	Deoxyribonucleic acid
EC	Electrical conductivity (measure of soil salinity)
GC%	Guanine-cytosine content (DNA base composition)
INRAA	National Institute of Agronomic Research of Algeria
IRT	Inhibitor Removal Technology (DNeasy <sup>®</sup> PowerSoil <sup>®</sup> kit)
NGS	Next-generation sequencing
NPK	Nitrogen-phosphorus-potassium fertilizer
OC	Organic carbon
OCS	Organic carbon stock
OM	Organic matter
OTU	Operational taxonomic unit
PA	Amended plot (improved management practices)
PC	Conventional plot (mineral fertilization)
PCR	Polymerase chain reaction
PE	Paired-end sequencing
PT	Control plot (uncultivated since 1942)
QC	Quality control
Q20/Q30	Sequencing quality scores (1/100 or 1/1000 error rate)
SOC	Soil organic carbon
SSUrRNA	Small subunit ribosomal RNA (taxonomic annotation database)
UPGMA	Unweighted pair group method with arithmetic mean (clustering method)

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