Original Article

Soil Organic Carbon Dynamic and Microbial Interactions in The Arable Land of Duhok Governorate: Implications for Carbon Sequestration

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Abstract

This research aimed to determine the key role of microbial diversity, segetal plants, and location (edge versus center) in regulating the dynamics of soil organic carbon (SOC) in the agricultural lands of the Kurdistan region, Iraq. A key finding was that significant SOC dynamic variability exists in sites related to spatial site-specific conditions. A high degree of SOC variability was also recorded intra-site, with edge localities consistently having more SOC than central regions. This suggests that micro-environmental conditions or interactions with adjacent ecosystems may promote SOC accumulation in edge environments. Microorganisms such as Penicillium, Fusarium, and B. cereus were seen as contributing to the decomposition of organic matter, resource cycling, and soil health improvement. Complex interactions among the microorganisms' SOC content, with fungi such as A. quadrilineatus, negatively correlate with SOC and prevent carbon accumulation. Fungi such as A. sydowii and A. malleus positively correlated with bacteria, suggesting mutualistic relationships that support soil fertility and plant growth. Penicillium species, P. citrinum, and P. centrum support microbial collaboration, which is important for carbon storage. Conversely, F. incarnatum has negative correlations with various microbes, emphasizing its role in competition over SOC dynamics. However, while identifying several explanatory factors, the predictive model shows that much of the SOC variance is left unexplained, indicating the need for even more refined models that scale up to larger ecological processes. Therefore, these findings exemplify the importance of microbe interactions in managing SOC, soil health, and carbon cycling.

Keywords: carbon storage, microbial diversity, segetal plants, sustainable agriculture, biodiversity conservation

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Introduction

Segetal plants, non-crop plants that grow alongside crops, depend heavily on the soil's microorganisms for their development and well-being. By aiding in the decomposition of organic debris, the cycling of nutrients, and the prevention of soil-borne illnesses, microorganisms such as bacteria, fungi, and actinomycetes improve the conditions for segetal plants (37). These microbes increase the availability of essential nutrients, including potassium, phosphorus, and nitrogen, while also enhancing the fertility and structure of the soil (37). Additionally, symbiotic relationships between some soil bacteria and segetal plants

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help the plants absorb nutrients and endure stress (32). The sustainability and general biodiversity of agricultural ecosystems and the well-being of segetal plants depend on this microbial activity (1) .

The soil of segetal plants may include various microorganisms essential to soil health and development. The microbiological groups of bacteria, fungi, archaea, and actinomycetes are significant. Pseudomonas, Bacillus, and Rhizobium are widespread bacterial genera well-known for inhibiting disease and promoting fungi, including mycorrhizal species like Glomus and saprophytic fungi like Trichoderma. They increase nutrient availability and protect plants against illness, particularly those in the Streptomyces genus, and are crucial for the breakdown of complex organic compounds and the production of antibiotics that prevent diseases from spreading via soil (13). Although less well-known, archaea are starting to be acknowledged for their contributions to nitrogen cycling, especially in extremely harsh soil environments (34).

Using microorganisms and SOCs in various wheat fields as segetal plant soils of the Kurdistan region and the larger context of Iraq requires urgent attention. Segetal plants growing as companions to the crops rely on soil microorganisms for many functions, including nutrient cycling, organic matter decomposition, and disease suppression, to facilitate their growth and betterment of resilience (37). Particularly, the bacteria and fungi in these soils help enhance the availability of nutrients, which include phosphorus, nitrogen, and potassium, all of which are critical for the growth of these plants. However, studies specifically targeting the relationship between microorganisms and SOC in segetal plant soils in Iraq, especially in the Kurdistan region of Iraq, are scanty .

Global efforts have been undertaken to study the influence of microorganisms in promoting the health and growth of segetal plants and their ability to convert organic matter into nutrients available for their growth (32), although such research, especially concerning segetal plants, remains limited in Iraq. This is a significant gap since understanding how microbial communities interact with SOC in segetal plant soils provides valuable insight into how these plants develop adaptations to harsh conditions in arid and semi-arid environments like those of Iraq. In addition, such microbial communities in these soils, including Pseudomonas, Rhizobium, mycorrhizal fungi, and Trichoderma species, have been shown to enhance soil fertility and plant growth in other regions, but where those interactions occur has largely escaped detailed study within Iraqi ecosystems (34).

Also, there are soil-degrading threats in Iraq, including erosion and declining fertility, which jeopardize the future of segetal crops, including wheat. Studies of microbial-SOC interrelationships in the Kurdistan region of Iraq could contribute essential knowledge on soil health, enhance crop production, and promote biodiversity conservation. Therefore, this study examines the relationship between soil microorganisms (i.e., bacteria and fungi) and SOC in wheat fields and segetal plant soils in Iraq. Specifically, it addresses whether SOC dynamic variability is related to spatial site-specific conditions, and whether both segetal plants and location (edge versus center), as well as microbial diversity (bacteria and fungi) contribute to SOC dynamics.

Materials and Methods

This study was conducted at nine different arable lands in Duhok province in the Kurdistan region of Iraq (Table 1). Situated in the Upper Mesopotamia Region, Duhok is mainly characterized by its remarkable natural and agricultural landscape diversity, i.e., mountains, valleys, plains, orchards, crops, and arable lands. The province primarily comprises open oak forests with a dense covering of herbaceous vegetation on the ground. In the plains, almost all the areas have been used for a long time for agriculture proposes. The climate of Duhok province is continental Mediterranean, with warm, dry summers and moderate, rainy winters (24). With an annual rainfall of about 1100 mm, most precipitation occurs in late winter to early

spring. Summer temperatures are often below 40 °C while in winter they are below 1°C, with snowfall occurring regularly throughout the winter months. It falls within the Csasensu Koppen (warm temperate – summer dry – hot summer) category of the Köppen-Geiger bioclimatic system (24).

Soil samples were collected from three quadrats (1m * 1m) in each of the nine distinct sites (Table 1) within the study area. Four soil samples were taken from each quadrat at two designated positions, two each from the edge and the center, thereby representing all areas within the quadrat. The sampling depth of each soil sample was set at 0-30 cm as recommended by (12). For composite soil samples of each quadrate, the four samples (two each from the edge and the center) were mixed thoroughly, resulting in one composite from the edge and the center for each quadrate; hence, a total of six composite samples were collected per site, three each from the edge and the center.

The samples were placed in clean, airtight plastic bags to prevent moisture loss and contamination. Each sample was tagged with all necessary site identification information, including site name and quadrate position, ensuring easy tracking and identification during analysis. Then, the soil samples were refrigerated until their SOC and micro-organism diversity were analysed.

Table 1. Study sites with average of rainfall and temperature in Duhok Governorate

No.	Site	Latitude (N)	Longitude (E)	Rainfall (mm)	Temperature (ºC)
1	Bardarash	36.3102	43.3546	409.72	21.95
2	Akre	36.4201	43.4748	849.9	20.24
3	Deralok	37.0323	43.4058	763.74	20.82
4	Sharya	36.8095	42.9532	619.49	20.5
5	Chra	36.3752	43.3024	312.6	25.99
6	Zakho	37.1106	42.5540	680.7	27.9
7	Mangesh	37.0301	43.1625	873.4	19.86
8	Sumail	36.8624	42.8666	453.35	20.88
9	Sumail - Kwashe	36.5543	42.4552	453.35	20.88

***Drying and sieving the soil samples**:* Organic carbon analysis was conducted for soil. After the soil samples were collected and transported to the laboratory for analyzing SOC, they were placed in an oven at 40 °C to 60 °C for hours until they dried. The soil samples were prepared for adequate SOC assessment by removing soil humidity through this dryness process.

The Walkley-Black method was used to measure SOC. One gram of air-dried soil (0.15 mm) was weighed and placed in a 500 mL beaker. Then, 10 mL of N potassium dichromate solution was added using a pipette, followed by adding 20 mL of concentrated H_2SO_4 with a dispenser. The beaker was swirled to mix the suspension and the solution was allowed to stand for half an hour. Next, 200 mL of deionized water and 10 mL of concentrated H_3PO_4 were added using a dispenser, and the mixture was left cool, following which 10–15 drops of diphenylamine indicator was added, a Teflon-coated magnetic stirring bar was inserted in a beaker and placed on a magnetic stirrer. The solution was titrated with 0.5 M ferrous ammonium sulfate until the color changed from violet-blue to green. Two blanks containing all reagents but no soil were prepared and treated as soil suspensions.

The SOC was calculated using the following equation:

$$M = 10/V \text{ blank}$$

$$\text{Oxidizable organic carbon (\%)} = (V_{blank}-V_{sample}) * 0.3 * M/Wt$$
$$\text{Total organic carbon (\%)} = 1.334 * \text{Oxidizable organic carbon (\%)}$$

Where:
M = molarity of $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution (about 0.5 M)
V blank = volume of $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution required to titrate the blank (mL)
V sample = volume of $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution required to titrate the sample (mL)
Wt = weight of air-dry soil sample (g)
$0.3 = 3 * 10^{-3} * 100$, where 3 is the equivalent weight of C

Microbial isolation and culturing: The collected soil sample was first sieved through a 2-mm sterile mesh to rid it of all large debris, stones, and roots. A total of 10g of the sieved soil was weighed in a sterile 250-milliliter Erlenmeyer flask and supplemented with 90 milliliters of sterile phosphate-buffered saline (PBS) or distilled water to effectuate a 1:10 dilution (9). The suspension was shaken at 150 rpm for 30 minutes to free the microorganisms from the soil into the liquid phase. For serial dilution, 1 ml of the soil suspension was transferred into a sterile test tube with 9 ml of sterile PBS and distillate, making it a 1:10 dilution (10^{-1}). The mixture was vortexed well, after which this was repeated as necessary, creating further dilutions (10^{-2}, 10^{-3}, 10^{-4}, etc.).

Different agar media were administered for different characteristics of the microorganisms in the study for their bacterial and fungal cultivation. Nutrient agar (NA) was applied for general bacterial growth, while potato dextrose agar (PDA) was used for fungal isolation. Some selective media, such as actinomycete isolation agar or mannitol salt agar, were also applied for particular bacterial groups. A total of 100 µL pipetting with each dilution was done into sterile agar plates and evenly distributed by a sterile spreader. In enrichment studies, soil suspensions were also plated on enrichment media. Virgin bacterial plates were kept inverted and incubated at temperatures based on the species, ranging from 28 to 37 °C for 24 to 72h. For the incubation of fungal plates, conditions of 25 to 30 °C, where growth was tested daily for durations from 3 days to 2 weeks inside the dark or slightly lighted areas, were provided.

Microscopic examination: Fungi species identification was made at the laboratories of the College of Agricultural Engineering Science, University of Duhok. A small piece of fungal mycelium or spores was mounted on a glass slide with a drop of lactophenol cotton blue stain. A coverslip was placed over the sample, and the slide was examined under a microscope. Morphological identification was performed based on spore structures, hyphal morphology, and reproductive features, utilizing taxonomic keys for classification.

Identification of bacterial species: After a soil sample was cultured on nutrient agar and based on morphological characteristics, different colonies were selected and aseptically transferred to buffered peptone water. These cultures were incubated for 24 hours at 37 ºC. From this, 1-2 ml of the harvested broth was used for genomic DNA extraction. The AddPrep Bacterial Genomic DNA Extraction Kit (Add bio, South of Korea) was used following the company guide. The quality and quantity of the extracted DNA were checked with the NanoDrop 2000/2000c (ThermoFisher Scientific, USA) and agarose gel electrophoresis (2). A set of previously published primer (Forward 5'GGAACTGAGACACGGTCCAG'3, Reverse 5'CCAGGTAAGGTTCTTCGCGT'3) was used to amplify a partial region of 16S rRNA (660 bp) for bacterial confirmation. Altogether, 50µ of the PCR reaction was made containing 25µ of 2X AddStart Taq Master (Add bio, South Korea), 2µ of each forward and reverse primers (10 pmol/µ), 5µ of genomic DNA, and the volume was adjusted by nuclease-free water. The heating program was set up in an applied biosystems thermal cycler (ThermoFisher Scientific, USA) as follows: one cycle of initial denaturation at 94

ºC for 5 minutes, 35 cycles of denaturation at 94 ºC for 0.5 minutes, annealing at 57 ºC for 0.45-minute, extension at 72 ºC for 1.30 minute, followed by one cycle of final extension at 72 ºC for 7 minutes. The results were confirmed in 1.5% agarose gel electrophoresis (Figure 1). 30µ of confirmed samples for partial 16S rRNA were used in sequencing in both directions (forward and reverse) by Macrogene, South Korea.

***Statistical analysis**:* The data was analyzed using SPSS software (33), including descriptive statistics, two-way ANOVA, principal component analysis (PCA), and linear regression analysis. The main five factors related to total SOC were identified, including Center/Edge, *Fusarium incarnatum*, *Penicillium centrum*, *Penicillium simplicissimum*, and *Bacillus cereus*. A prediction equation for total SOC was derived using linear regression and correlation analysis, with Duncan's multiple range test separating the means.

Results and Discussion

The result showed that the primers used in the PCR reaction could amplify the targeted 660 bp of 16S rRNA from isolated bacteria (Figure 1). After sequencing these amplicons, the blast results showed that these bacteria isolates belong to *Staphylococcus sp, Bacillus subtilis, Bacillus cereus, Acidobacteria bacterium,* and *Staphylococcus* sp sub. (*Aureus* and *Pasteur*). The fungi and bacterial speciess of each site are shown in Table 2.

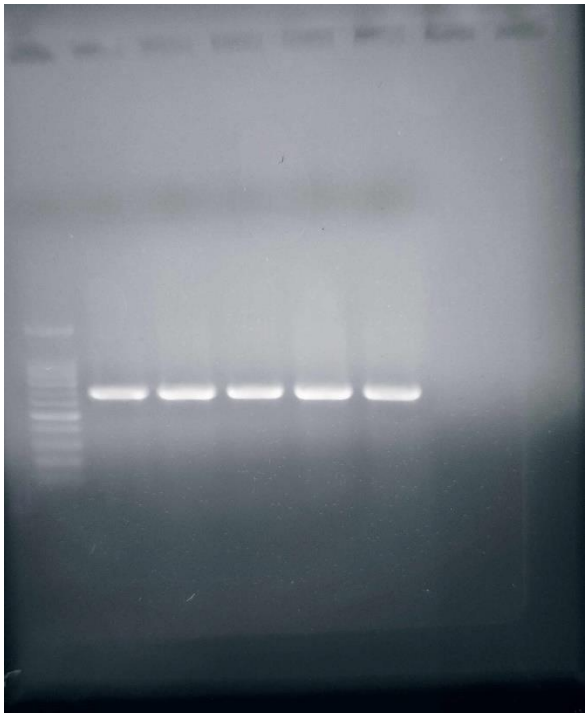

Figure 1. Agarose gel electrophoresis (1.5%) of amplified 16S rRNA from isolated bacteria
Lane 1: 100 bp DNA ladder; lanes 2-5: PCR products of partial 16S rRNA (660 bp)

Table 2. Microbial fungi and bacteria diversity in each site

Site	Fungi Species	Bacteria Species
Bardarash	Aspergillus niger Aspergillus sydowii	Staphylococcus sp. Bacillus subtilis Bacillus cereus Acidobacteria bacterium Staphylococcus spp. sub. (Aureus and Pasteur)
Akre	Aspergillus niger Aspergillus sydowii Aspergillus quadrilineatus	Staphylococcus sp. Bacillus subtilis Bacillus cereus Acidobacteria bacterium
Deralok	Aspergillus niger Aspergillus melleus Aspergillus sydowii Penicillium citrinum	Staphylococcus sp. Bacillus subtilis Bacillus cereus Staphylococcus spp. sub. (Aureus and Pasteur)
Sharya	Aspergillus niger Aspergillus sydowii Fusarium incarnatum	Staphylococcus sp Bacillus subtilis
Chra	Aspergillus niger Aspergillus sydowii Penicillium centrum Penicillium simplicissimum	Bacillus subtilis Bacillus cereus Staphylococcus spp. sub. (Aureus and Pasteur)
Zakho	Aspergillus niger	Bacillus subtilis Bacillus cereus Staphylococcus spp. sub. (Aureus and Pasteur)
Mangesh	Aspergillus niger Penicillium simplicissimum	Bacillus subtilis Bacillus cereus
Sumail	Aspergillus niger Aspergillus quadrilineatus	Staphylococcus sp. Bacillus subtilis Bacillus cereus Ochrobactrum anthropic
Sumail - Kwashe	Aspergillus niger Fusarium incarnatum	Bacillus subtilis Bacillus cereus

Total mean SOC across all sites: The first set of statistical analyses on the total mean SOC across all sites showed it to be 0.653 ± 0.003, with a 95% confidence interval ranging from 0.647 to 0.658 (Table 3). This indicates a moderate SOC level, providing a reference point for evaluating site-specific variations. The above SOC value of all the sites can be classified as moderate soil organic matter. This finding agrees with almost all cases in dryland ecosystems, where bulk storage of SOC to 40 cm depth was reported to be 1,922.38 teragrams, with a SOC density gradient from southwest to northeast (44). This relatively moderate SOC level can be explained mainly by climate factors of arid lands (Table 1).

In fact, the low precipitation limits plant biomass production and subsequently determines organic matter input to soils of drylands (5). However, better land management practices such as conservation tillage, cover cropping, and organic amendments increase SOC levels. (43) noted that sustainable land management will induce soil carbon accumulation and more soil fertility, which helps carbon sequestration. Since soils with improved management practices have the potential to sequester 0.28 to 0.43 gigatons of carbon per year (43), the present SOC level of the study sites, therefore, provides an excellent basis for implementing carbon-sequestration-enhancing endeavors that could be aligned with global standards. Recent evidence further indicates that soil organic carbon stabilization is strongly mediated by microbial mineralization

responses to temperature and management-driven changes in carbon inputs (41).

With mean values ranging from 0.140% to 1.162%, significant differences in SOC were noted between locations ($P < 0.01$). The highest SOC was 1.162% for Bardarash, 1.065% for Mangesh, and 0.850% for Sumail-Kwashe. Akre had the lowest SOC value of 0.140% while Chra and Deralok followed with SOC values of 0.538% and 0.387% respectively according to (Table 3). Multiple primary elements determine SOC levels across different sites which vary due to differences in terrain and plant cover and climate conditions and soil management methods. The study suggests that Bardarash and Mangesh provided better environmental conditions for SOC formation because they received larger amounts of organic matter or experienced more persistent environmental conditions. The site shows higher SOC values which confirm earlier research results that organic carbon application improved soil structure and fertility while increasing microbial biomass (15) moreover (42) in their study shows the differences in plant- versus microbial-derived carbon inputs can strongly influence the persistence of SOC and its potential for carbon sequestration in cropland and dryland soils.

Bardarash maintains a rich microbial population that includes fungus like *Aspergillus niger* and *Aspergillus sydowii*, as well as bacteria like *Staphylococcus* sp., *Bacillus subtilis*, *Bacillus* cereus, *Acidobacteria bacterium*, and *Staphylococcus* sp. sub. Aureus and Pasteur (Table 2). Sumail's soil fertility is enhanced by the diverse microbial species, which contribute to carbon sequestration and nutrient cycling, resulting in high soil fertility and optimal organic carbon retention. This diversity is beneficial for soil health as it aids in organic matter decomposition, improving nutrient cycling and soil stability (1). However, the sites in Akre and Deralok (0.140%, 0.387%, respectively) have poor SOC values despite having *Aspergillus niger* and *Aspergillus sydowii* as well as bacteria like *Bacillus subtilis* and *Staphylococcus* sp.

These conditions of exhibiting active decomposers of microbial community may lead to higher organic carbon reduction in decomposition or reduced SOC storage. (7) reported that the highland regions of northern Iraq, involving Deralok and Akre, received more than 1,000 mm of rain every year (Table 1), a level of precipitation less than what both sites require. Environmental factors such as pericipitation, high temperatures, soil disturbance, and poor vegetation can accelerate the breakdown of organic carbon (8) as well as most of the *Fusarium* spp, *Aspergillus* spp, and *Penicillium* spp which are saprophytic and receive their energy from breaking down dead organic matter (1). *Staphylococcus* spp. can be found in soils having a lot of organic matter, but they aren't usually the ones that break down soil. Without knowing which subgroups of *Acidobacteria* are present, it is difficult to say what their role is. *Bacillus* spp., on the other hand, are well-known saprophytes in arable soils actively encouraging the constant turnover of organic matter (1), which lead to low organic carbon values in certain sites. *Aspergillus* species that decompose organic matter quickly turnover organic material, causing a depletion of SOC levels.

The outcome of the interplay with climate, soil texture, and vegetation, generally results in disturbed ecosystems having poor microbial diversity and carbon storage (9). The climatic variables, temperature, and precipitation jointly impact organic matter since they influence plant growth and decomposition rates (15). Increased vegetation cover results in higher organic matter input and, in contrast, limited cover at low rainfall and high temperatures reduces organic matter input (15). Different plant species release organic matter in varying amounts and types, thus determining the carbon content in soil. Soil management practices such as tillage, use of fertilizer, and crop rotations may affect SOC content (2). By associating conservation tillage with organic amendments, SOC sequestration can be enhanced, while overstocking is considered a depletion (25).

Topography, regarding slope and slope aspects, affects the movement and retention of water. This landscape factor alters the types of vegetation and subsequently influences SOC levels. Generally, areas with the most favorable topographic features will be covered by dense vegetation, giving rise to retention of moisture in the soil and, thus, enhancement of SOC stock (39). Also, topography, ecology, erosion, and soil moisture have a multitude of interactions by which carbon could be sequestered, thus indicating how complex the relationships among these vectors are (16). Therefore, these differences in SOC between locations were most likely due to a complex interplay of climatic conditions, vegetation types, soil management practices, and topographic characteristics.

Though precipitation is necessary for plant growth and as a source of organic carbon input, temperature and soil disturbance play a more important role in carbon cycling than the other factors (8). High temperatures hasten organic carbon decomposition so that little capacity will remain for carbon to be stored (30), similar to the low SOC values at Akre and Deralok noted as this research was conducted between summer and autumn 2024 when the temperatures were high, at between 34 ^{o}C to 40 ^{o}C according to met data in Duhok. Soil erosion or tillage, increase the exposure of organic carbon and promote its earlier microbial decomposition, aggravating the loss from the soil of sequestered carbon (15). Soil textures also affect SOC content, with their clay particle structures having a greater ability to protect organic carbon from attacks by microorganisms, as with organic carbon forms in sandy soils which have larger particles. The microbial communities in these soils interact with environmental factors and have a role in organic carbon decomposition and influence nutrient cycling, contributing to soil health, well-being, and ability to sequester carbon.

Observations of the microbial communities across the various sites reveal a unique role in organic carbon decay and nutrient cycling. Such fungi as *Aspergillus* species, which predominated in most of the sites, are recognized as decomposers that break apart as much organic complex materials as possible, thus significantly contributing to nutrient cycling and carbon storage (3). Microbial activity at Sumail, a location with high microbial diversity, would imply carbon sequestration efficiency by decomposing organic carbon at a balanced rate. Soil improvements made through this method store carbon, increasing soil fertility. However, higher fungal activity than organic carbon input may cause faster turnover of organic material in locations with lower organic carbon inputs, like Akre and Deralok, resulting in lower levels of SOC (3).

The key role of segetal plants and edge versus center location on SOC: Native segetal plant species in fields may have potential for carbon sequestration due to microbial activity levels held under environmental conditions. Bardarash and Mangesh's sites are suitable for an optimal condition of such carbon, where various fungal and bacterial communities help in carbon sequestration and nutrient cycling (15). Meanwhile, Akre and Deralok, have lower SOC mainly due to high decomposition rates or low organic carbon inputs caused by environmental factors such as temperature, soil disturbance, and vegetation cover (8). It does seem that segetal plants can sequester carbon in the soil, but the extent to which this happens depends greatly on site-specific conditions and microbial dynamics (3). A significant difference was observed between the center and edge locations ($P < 0.01$). SOC was higher at the edge (0.825%) compared to the center (0.481%) (Table 3). At different levels of SOC, the edge-center difference seems significant enough to suggest the possibility of spatial variability.

Various reasons can be attributed to the differences, including variations in micro-climates, vegetation cover, or cultural practices with the soil. Effectively, it has been suggested that some forest edge effects play a role in determining soil properties, including organic carbon content, by temperature and moisture changes and plant diversity (4). Studies show that edge locations tend to have increased soil respiration rates with no

corresponding change in soil carbon stocks, thus affirming the complex dynamics at these interfaces (27). Thus, the higher SOC in this study's edge locations might be due to factors underlying the importance of spatial variability in considering soil carbon dynamics as well as changes in the structure of a community or population at the border of two habitats. Living at these edges often means that organisms have different environmental conditions (lighter, moisture, and nutrients) and more resources available to them (28).

Interaction between site and location: This further supports the observed SOC trends. All the edge areas at Bardarash, Mangesh, and Sumail-Kwashe exhibited high SOCs at 1.614%, 1.291%, and 1.054%, respectively, while low SOC values were recorded in the center areas of Akre (0.108%) and Deralok (0.301%). Additionally, sites such as Sumail and Sharya demonstrate a notable increase in SOC at the edge compared to their center locations, indicating a pattern where edge environments favor SOC accumulation. However, sites like Chra and Zakho, showed a minor increase in SOC between the center and edge. Thus, the high spatial variability of SOC between the two locations at several sites implies that factors influencing carbon storage may include topographical, environmental, and likely anthropogenic ones.

Higher SOC values were consistently found at the edge locations of Bardarash (1.614%), Mangesh (1.291%), Sumail-Kwashe (1.054%) compared to the centers of Akre (0.108%) and Deralok (0.301%) (Table 3), corroborating findings of previous studies that reported enhanced organic carbon stocks in edge environments (5). This may occur more in a situation where the environment thrives with a rich diversity of vegetation, thereby enhancing root biomass and an equal stabilization of soil conditions (6). Also, the lower SOC observed in those center localities, especially Akre and Deralok, could be attributable to a host of issues ranging from reduced vegetation cover to diminished organic matter inputs to possible soil degradation through heavy grazing, tillage, or erosion, all of which a contributing factor to SOC depletion (15).

These low values are indeed below the global average for SOC, which is generally quoted as between 1% and 5% for natural soils (28). Still, some regions, particularly in the tropics and temperate zones, with low disturbance, have been reported to have much higher SOC concentrations, often up to 10% or more under forest or wetland conditions (29). Therefore, the results from Sumail and Sharya confirm that high SOC at the edge compared to the center is strongly influenced by exogenous factors like microclimatic differences, topography, and vegetation cover. On the contrary, the work from sites like Chra and Zakho, where only slight SOC differences occur between the edge and the center, suggests that local environmental factors such as soil texture or land management may have diluted the edge effects (21).

Thus, from the perspective of global standards, SOC concentrations in this study, particularly the high ranges such as Bardarash, generally fit the values found in carbon-rich ecosystems. On the other hand, some low values noted in various sites imply that the potential for SOC sequestration increases with better land management practices. Therefore, in strategizing for improving carbon storage, mitigating climate change, and enhancing soil health in severely degraded regions, understanding the environmental drivers that promote SOC distribution is of critical importance.

Table 3. Effect of site, location, and their interaction on total SOC

Overall Mean				
Overall Mean	**Mean**	**± Std. Error**	**95% Confidence Interval**	
			Lower Bound	**Upper Bound**
Total SOC	.653	.003	.647	.658
Impact of Site				

Site	Mean	± Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Akre	.140 h	.008	.123	.157
Bardarash	1.162 a	.008	1.145	1.179
Deralok	.387 g	.008	.370	.404
Chra	.538 f	.008	.521	.555
Mangesh	1.065 b	.008	1.048	1.082
Sharya	.645 d	.008	.629	.662
Sumail	.570 e	.008	.553	.587
Sumail - Kwashe	.850 c	.008	.833	.867
Zakho	.516 f	.008	.500	.533
Sig. (p-value)		P<0.01 **		

Impact of Location (Center/Edge)

Center /Edge	Mean	± Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Center	.481 b	.004	.473	.488
Edge	.825 a	.004	.817	.833
Sig. (p-value)		P<0.01 **		

Interaction

Site	Center / Edge	Mean	± Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Akre	Center	.108 k	.012	.084	.131
	Edge	.172 j	.012	.148	.196
Bardarash	Center	.710 e	.012	.686	.734
	Edge	1.614 a	.012	1.590	1.637
Deralok	Center	.301i	.012	.277	.325
	Edge	.473 h	.012	.450	.497
Chra	Center	.452 h	.012	.428	.476
	Edge	.624 f	.012	.600	.648
Mangesh	Center	.839 d	.012	.815	.863
	Edge	1.291 b	.012	1.267	1.315
Sharya	Center	.516 g	.012	.493	.540
	Edge	.775 e	.012	.751	.798
Sumail	Center	.366 i	.012	.342	.390
	Edge	.775 e	.012	.751	.798
Sumail - Kwashe	Center	.646 f	.012	.622	.669
	Edge	1.054 c	.012	1.031	1.078
Zakho	Center	.387 i	.012	.364	.411
	Edge	.645 f	.012	.622	.669
Sig. (p-value)		P<0.01 **			

Relationship between SOC, bacteria, and fungi species: This study used principal component analysis (PCA) to identify the main factors influencing SOC based on 16 factors. Five factors were chosen as predictors for total SOC in the future, including *Aspergillus niger* fungi and *Bacillus subtilis* bacteria, which were found in all sites and locations (Figure 2). This suggests that adaptable microbes display a specific baseline microbial activity without necessarily acting as drivers of SOC variability across different ecosystems (17). Microbial diversity and functional traits are more important for SOC dynamics than merely being present or abundant (11). Different microbial communities utilize carbon differently, hence serving as pivotal determinants in SOC storage potential and accumulation and decomposition processes. Shifts in microbial functional groups better

counter SOC predictions (35). The five selected likely factors represent microbial-environmental interactions responsible for SOC trends over time, requiring the inclusion of only microbial aspects for soil carbon modeling.

Figure 2 shows that the center/edge, *Penicillium centrum* fungi, *Fusarium incarnatum* fungi, *Penicillium simplicissimum* fungi, and *Bacillus cereus* bacteria, are the most clustered factors. Therefore, the prediction equation is derived and developed depending on only the mentioned factors. Such clusters are corroborated by earlier studies suggesting that conspecific microbes with similar ecological functions and adaptations to their environment are forced to co-exist due to niche differentiation and synergistic interactions (11). The spatial organization of microbes is important for SOC dynamics because closely bound microbial groups share metabolic pathways in carbon cycling (11).

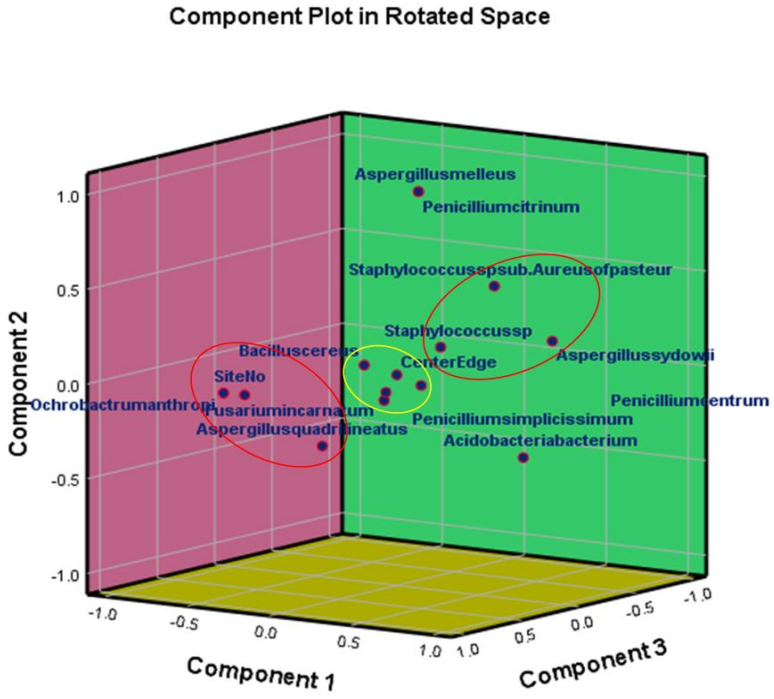

Figure 2. The clustered factors in 3D

The genera *Penicillium* and *Fusarium*, known decomposers, build up organic matter with the aid of extracellular enzymes and hence contribute to nutrient cycling and stabilization of SOC (35). Thus, *Bacillus cereus* can promote carbon turnover by hydrolyzing complex carbon substrates into simpler ones through its enzymes and thereby participating in the formation of stabilized soil carbon fractions (17). Such associated clustering of these taxa suggests functional interdependency that would directly affect SOC dynamics. There are good reasons to argue that an SOC prediction equation should be implemented, considering strong ecological implications and spatial association. This reinforces the discussion on favoring microbially functional traits for better predictability and ecological relevance, rather than just their presence in soil carbon models.

Moreover, it may derive a new prediction equation according to two main factors that increase confidence and reliability. The chosen factors were Center/Edge and *Bacillus cereus* (those were very close to each other and had low communalities).

The prediction equation:

SOC = 0.129 + 0.344 (Center/Edge) + 0.008 (*Bacillus cereus present*)

Table 4 shows the model's summary of the outputted equation. The neglible R^2 value of 0.217 suggests that a considerable proportion of the variability in SOC remains unexplained. The study encompasses segetal plants, soil bacteria, fungi, and space differences (edge vs. center), all considered influential factors in SOC. However, the more important factor considerations are those of a biotic or abiotic nature or their interaction in a very complex way. Some of these soil properties include texture, pH, moisture content, and bulk density, which are paramount in stabilizing carbon and the activity of microorganisms (31). Temperature and precipitation conditions in a particular place control the dynamics of biological activity by facilitating or retarding their decomposition rate and then affecting their accumulation in organic matter, thereby affecting SOC storage (36).

Table 4. Model summary of the prediction equation

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.466[a]	.217	.186	.3367598	.217	7.056	2	51	.002

a. Predictors: (Constant), Bacillus cereus, Center/Edge

Dependent Variable: Total SOC

Land-use and management practices such as tillage, chemical fertilization, or crop rotation generally modify the carbon inputs and change the dynamics of microbial populations, thus varying SOC retention (23). Plant litter quality, quantity, and root biomass can also be considered belowground inputs of carbon (21), while erosion and sediment deposition have been cited as contributing to the redistribution of organic material within the soil profile (26). All types of soil fauna, including earthworms and other bioturbators, modify SOC cycling through organic matter breakdown and redistribution (15). The current model lacks the abovementioned crucial determinants and must, therefore, require an R^2 that is low for a wider approach integrating soil physicochemical properties, climatic conditions, and land management strategies required to enhance SOC predictions. Indeed, studies conducted in similar domains reported low R^2 due to the complexity of inherent SOC dynamics.

Thus, (20) found that despite including several soil properties, their model explained only about 20% of SOC variance when tested against external observations. Similarly, in a study on soil organic matter by (38), an R^2 value of 0.25 was derived, indicating that much of the variance was unexplained with those variables included. These aspects quite well illustrate the complexity and multifactorial nature which shape SOC dynamics. Increasingly, microbial activity, biodiversity of interactions in the rhizosphere including rhizoplankton, extra-rhizosphere zone, and root-microbe associations are being recognized as key drivers for carbon sequestration and loss (2).

Correlation coefficients were computed to determine the relationship between the studied variables (Table 5). Both Pearson correlation analyses were done, and it appeared that total SOC just correlated negatively and significantly ($p<0.01$) with *Aspergillus quadrilineatus*, where SOC value is decreased when the mentioned fungi are present in the soil. The rest of the coefficients between fungi and bacteria are significant ($p<0.05$), meaning the presence of a relationship (some positive and others negative) between those fungi and bacteria.

Table 5. Correlation coefficient between the studied variables

	Total SOC	Aspergillus sydowii	Aspergillus quadrilineatus	Aspergillus melleus	Penicillium citrinum	Fusarium incarnatum	Penicillium centrum	Penicillium simplicissimum	Staphylococcus sp	Bacillus cereus	Acidobacteria bacterium	Staphylococcus sp sub. Aureus of pasteur	Ochrobactrum anthropi
Total SOC	1	-.236	-.430**	-.254	-.254	.137	-.110	.215	-.217	.007	-.003	-.004	-.079
Aspergillus sydowii	-.236	1	-.060	.316*	.316*	-.060	.316*	-.060	.550**	-.316*	.478**	.350**	-.395**
Aspergillus quadrilineatus	-.430**	-.060	1	-.189	-.189	-.286*	-.189	-.286*	.478**	.189	.357**	-.478**	.661**
Aspergillus melleus	-.254	.316*	-.189	1	1.000**	-.189	-.125	-.189	.316*	.125	-.189	.395**	-.125
Penicillium citrinum	-.254	.316*	-.189	1.000**	1	-.189	-.125	-.189	.316*	.125	-.189	.395**	-.125
Fusarium incarnatum	.137	-.060	-.286*	-.189	-.189	1	-.189	-.286*	-.060	-.661**	-.286*	-.478**	-.189
Penicillium centrum	-.110	.316*	-.189	-.125	-.125	-.189	1	.661**	-.395**	.125	-.189	.395**	-.125
Penicillium simplicissimum	.215	-.060	-.286*	-.189	-.189	-.286*	.661**	1	-.598**	.189	-.286*	.060	-.189
Staphylococcus sp	-.217	.550**	.478**	.316*	.316*	-.060	-.395**	-.598**	1	-.316*	.478**	-.100	.316*
Bacillus cereus	.007	-.316*	.189	.125	.125	-.661**	.125	.189	-.316*	1	.189	.316*	.125
Acidobacteria bacterium	-.003	.478**	.357**	-.189	-.189	-.286*	-.189	-.286*	.478**	.189	1	.060	-.189
Staphylococcus sp sub. Aureus of Pasteur	-.004	.350**	-.478**	.395**	.395**	-.478**	.395**	.060	-.100	.316*	.060	1	-.316*
Ochrobactrum anthropic	-.079	-.395**	.661**	-.125	-.125	-.189	-.125	-.189	.316*	.125	-.189	-.316*	1

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

This study involved a correlation analysis on SOC and several fungal and bacterial species in soil. Various degree correlations, both positive and negative, were found indicating the complex nature of the microbial interactions and associated dependencies on organic matter available in soil (19). Thus, these findings emphasized the nature of the soil ecosystems, where beneficial and competitive interactions depend on the specific conditions and species of microbes involved. Most curious is the negative linear relationship of SOC with *Aspergillus quadrilineatus* ($r = -0.430$, $p < 0.01$), signifying that the share of this fungus becomes less prevalent with increasing values of SOC.

This outcome may be explained in terms of the ecological habitat preferences of *A. quadrilineatus*, where conditions may be beneficial for this species in environments poor in nutrients where organic carbon is less plentiful. Higher amounts of SOC could produce more fungi or a community of microbes that could outcompete *A. quadrilineatus*, thus leading to its lower abundance in soils with higher SOC (40). These findings were corroborated in the research, indicating that soil fungi can show a certain degree of specialization according to the niche formed through the availability of organic matter and the other nutrients within soils that would generally determine their

presence and abundance in different kinds of soils (10).

In contrast, the remaining fungal species, like *Aspergillus sydowii* and *Aspergillus malleus*, showed positive correlations with bacteria like *Staphylococcus* sp. ($r= 0.550$, $p < 0.01$) and other fungi like *Penicillium citrinum* ($r = 0.316$, $p < 0.05$) respectively. Such positive combinations would suggest that some fungi and bacteria probably share a favorable ecological niche, often derived as a result of mutualistic or synergistic interactions. For instance, bacteria degrade organic matter into more readily susceptible forms, enabling fungi that depend on such decomposed material to benefit from it (22). Such an observation is usually recorded in the systems of soils, with fungi and bacteria working in cooperation for nutrient cycling and organic matter decomposition while lending mutual support to each other for growth (44). In addition, one of the critical means that allow earthworms to create healthy soils is through promoting mutual interactions between bacteria and fungi, thereby enhancing plant nutrient availability and mineralizing the breakdown of complex organic compounds (18).

Furthermore, the strong positive correlation ($r = 1.000$; $p < 0.01$) between *Penicillium citrinum* and *Penicillium centrum* indicates that both penicillia might have a congruent ecological role or an environmental preference. Also, fungi in the *Penicillium* genus have been shown to exhibit similar patterns of microbial co-occurrence due to their role in breaking down organic matter in high-carbon soils (38). On a collective basis, the species provide crucial functions in the larger soil fungal microbiome in nutrient cycling, mainly from their ability to decompose organic materials and maintain microbial diversity (23).

There was a negative correlation between certain bacteria, such as *Fusarium incarnatum* and *Bacillus cereus*, with the fungi, for example, *Staphylococcus* sp. ($r = -0.661$, $p < 0.01$). This suggests competitive exclusion, whereby some bacteria and fungi may be competing for similar resources like nutrients or space. In soils with high competition from microorganisms, some bacterial species have been reported to inhibit the growth of fungi through antimicrobial compounds (14). The competitive interactions in this study find agreement with the well-documented phenomenon of resource competition in microbial communities, wherein different species compete for nutrients, affecting their abundance and distribution in the soil ecosystem (14).

The overall correlation patterns in this study depict the dynamic and intricate interactions between soil microbes and organic matter content, moderated by environmental conditions, resource competition, and mutualism. For instance, higher SOC levels would favor certain fungi and bacteria adapted to the decomposition of organic materials, which can then modulate the composition of microbial communities (40). These negative relationships suggest that more organic matter favor certain microbes which alter community structure (18). Changes in the microbes are important to soil health because the their composition will vary with consequences in nutrient cycling, disease suppression, and the general functionality of the ecosystem (37).

Conclusion

Soil microbiomes, as the study indicates, are crucial in stabilizing SOC levels, particularly in Duhok province in Iraq. Micro-environmental conditions or interactions with adjacent ecosystems make edge environments amenable for SOC accumulation. Microbial factions including *Penicillium*, *Fusarium*, and *Bacillus cereus* are key in modifying SOC, cycling nutrition, and degrading substances, thus promoting soil health. *Aspergillus quadrilineatus* shows some negative relationship with SOC while fungi such as *Aspergillus sydowii* and *A. malleus* favor other bacteria reciprocally. There is potential for microbes to cooperate in favor of soil health and carbon storage.

Fusarium incarnatum is determined as having a negative correlation with microbes, thus posing itself as an antagonist negatively influencing SOC dynamics. A predictive

model states that much variability in SOC remains poorly understood; in fact, many biotic and abiotic factors combine to determine SOC, thus warranting better predictive models to help understand the more extensive ecological processes influencing SOC dynamics. Managing the balance between cooperation and competition among microbes is essential to soil health and carbon cycling.

An understanding of how microbial communities function to sequester carbon goes a long way toward solving issues related to climate change and environmental sustainability. Future research in Duhok must focus on ways to sequester more carbon in soils and practices aimed at making farming more sustainable. Introducing soil microbes from Bardarash that are richer into new soils like Deralok and Akre could possibly improve SOC levels. This intervention might even improve soil health and activity. However, this aspect requires further scientific investigation. Long-term efforts in this regard could result in sustainable agriculture for the region.

Supplementary Materials
None.

Author Contributions
All authors participated in the methodology, writing—original draft preparation, writing—review, and editing of this paper. They have read and agreed to the published version of the manuscript.

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Informed Consent Statement
No Informed Consent Statement.

Data Availability Statement
None.

Conflicts of Interest
The authors declare no conflict of interest.

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مقالة أصيلة

حركة الكربون العضوي في التربة والتفاعلات الميكروبية في الأراضي الصالحة للزراعة في محافظة دهوك: تداعيات على احتجاز الكربون

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الخلاصة

الحقلية في منطقة كردستان، العراق. لوحظت درجة عالية من التباين في قياسات الكربون العضوي في التربة، حيث كانت المواقع المحيطية تحتوي دائمًا على كربون عضوي أكثر من المناطق المركزية. يشير ذلك إلى أن الظروف الميكروكلينية أو التفاعلات مع النظم البيئية المجاورة قد تعزز تراكم الكربون العضوي في بيئات الحافة. تُبرز الدراسة أيضًا احياء مجهرية التربة مثل Penicillium و Fusarium و Bacillus cereus كمساهمين في تحلل المادة العضوية ودورة الموارد وتحسين صحة التربة. ترتبط التفاعلات المعقدة بين احياء مجهرية التربة بمحتوى الكربون العضوي، الا أن بعض الفطريات مثل Aspergillus quadrilineatus ترتبط ارتباطًا سلبيًا مع الكربون العضوي في التربة وقد تمنع تراكم الكربون. على العكس من ذلك، فإن الفطريات مثل *A. sydowii* و *A.* Melleus ترتبط ارتباطًا إيجابيًا مع البكتيريا، مما يشير إلى علاقات تكافلية تدعم خصوبة التربة ونمو النبات. فضلا عن ذلك ، تدعم أنواع Penicillium، و خاصة *P.* citrinum و *P.* centrum، التعاون الميكروبي المهم لتخزين الكربون. من ناحية أخرى، أظهرت *F.* incarnatum ارتباطات سلبية مع مختلف احياء مجهرية التربة، مما يبرز دورها في التنافس على ديناميكيات الكربون العضوي. ومع ذلك، بينما تم تحديد العديد من العوامل التفسيرية، يُظهر النموذج التنبؤي المُنشأ أن جزءًا كبيرًا من تباين الكربون العضوي في التربة لا يزال غير مفسر، مما يشير إلى الحاجة إلى نماذج أكثر دقة تأخذ في الاعتبار العمليات البيئية الأكبر. لذلك، تُظهر هذه النتائج أهمية تفاعلات احياء مجهرية التربة في إدارة الكربون العضوي في التربة، وصحة التربة، ودورة الكربون.

كلمات مفتاحية: تخزين الكربون، تنوع احياء مجهرية التربة، النباتات الحقلية، الزراعة المستدامة، حفظ التنوع الحيوي

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