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Soil Organic Carbon Fraction Dynamics and Morphological Responses of *Allium* Species under *Chlorella vulgaris* Application

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Abstract

This study investigated the impact of *Chlorella vulgaris* on the soil organic carbon (SOC) fractions and morphology of leek (*Allium porrum* L.*),* onion (*Allium cepa*), and garlic (*Allium sativum*). The experimental design followed a Completely Randomized Design (CRD) with three replications in a Greenhouse condition. Morphological parameters, including plant height, fresh and dry weight of the plant, root length, and fresh and dry weight of the root, were determined and evaluated using t-tests. No statistically significant difference was seen between the leeks treated with *C. vulgaris* and the control plants. Nonetheless, the leek plants subjected to *C. vulgaris* consistently exhibited superior mean values across all evaluated parameters. This indicated a favorable growth tendency; yet it lacked statistical significance. Onion plants and their roots showed distinct differences in fresh and dry weight. The control treatment produced more biomass than the *C. vulgaris* treatment, but other morphological traits remained unchanged. In garlic, the application of *C. vulgaris* significantly increased root length, indicating accelerated root development, while other morphological parameters remained unchanged. Soil carbon fractions responded more significantly to *C. vulgaris* application compared to plant morphology. *Chlorella* increased passive carbon (PC) levels and total SOC in leek soils by 37% and 33.7%, respectively. The soil under onions had the most significant response. They had a substantial increase in active carbon (AC), PC, and total SOC, and a decrease in intermediate carbon (IC). *Chlorella* treatment also caused significant rises in PC and SOC in garlic soils. The results indicated that *Chlorella* significantly influences soil carbon stability more than short-term plant growth, suggesting its potential to enhance soil carbon storage and improve long-term soil health.

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1. Introduction

People must eat a balanced, regular diet to maintain their lives (Uslu, 2022). In this regard, vegetable products are of great importance for individuals' health status because they contain high levels of micronutrients and have properties that help prevent and improve health-related problems (Eriş and Yanmaz, 1979; Sezgin, 2014; Uslu, 2022). Leek (*Allium porrum* L.), onion (*Allium cepa*), and garlic (*Allium sativum*) are edible plants belonging to the *Allium* species and are among the most consumed vegetables globally, considering their nutritional value and functions in living organisms (Bernaert et al., 2013; Golubkina et al., 2019; Uslu, 2022). Onion, within the Alliaceae plant family, is among the most essential vegetable varieties for agricultural activities in Türkiye (Hancı and Cebeci, 2015). The onion has a dense root system, which allows the plant to be grown immediately after with high levels of organic compounds (Beşirli et al., 2021). Garlic, also belonging to the Alliaceae plant family, is an annual vegetable with underground parts used for consumption, suitable for winter consumption, and of high importance (Köksal et al., 2014; Golubkina et al., 2019). It is a vegetable plant that has adapted to a wide range of global environments and is frequently cultivated (Köksal et al., 2014). Leek, a member of the Alliaceae family, is a winter vegetable suitable for consumption in Türkiye and is produced and consumed throughout the country (Parlak et al., 2021). Leek is a vegetable that can be grown year-round. However, when grown for seed production, it is a two-year-old herbaceous plant (Taşan and Altunkanat, 2019). Furthermore, the leek plant is rich in nutrients, containing elevated concentrations of carbon, nitrogenous organic compounds, lipids, dietary fiber, and minerals (Benas et al., 2024). Carbon, a fundamental component of life, has existed long after the formation of our planet and is found in the composition of all organic substances (Ekinci and Özer, 2025). The total carbon found in the soil is primarily composed of inorganic and organic carbon fractions. In regions with elevated moisture, soil carbon is of organic origin, but in locations with high temperatures, it is of inorganic origin (Bradford et al., 2008; Shaoxuan et al., 2016; Koyunçu, 2022). The carbon structure formed and stored as a result of the mineralization of plant and animal remains, which comprise a significant portion of the soil, is referred to as soil organic carbon (SOC) (Aşkın et al., 2014). SOC plays a crucial role in soil quality and in preventing soil degradation (Razzaghi, 2022a; Topçu et al., 2022). Soil, a vital component of local ecosystems, serves as both a carbon sink and a carbon source in the atmosphere (Yılmaz and Dengiz, 2021). Carbon sequestered in soils with optimal carbon storage capacity is crucial for maintaining the soil's physical, chemical, and biological characteristics, as well as its productivity and health, and the overall carbon cycle (Razzaghi, 2024a; Karamanlı, 2025). The augmentation of SOC levels not only contributes to the climate's equilibrium and mitigates declining water levels but also enhances soil structural properties, productivity, water retention capacity, the proliferation of beneficial microorganisms, and elevated plant productivity, as well as the efficient recycling of applied inputs (Rawls et al., 2003; Bronick and Lal, 2005; Wilson et al., 2009; Schmidt et al., 2011; Kane and Solutions, 2015; Evliyaoğlu, 2019). SOC is characterized by differing rates of persistence and decomposition, comprising both resistant and readily decomposable forms (Weil et al., 2003; Lehmann et al., 2020; Razzaghi et al., 2021; Amoakwah et al., 2022; Razzaghi, 2025). Islam and Weil (2000) indicated that while bulk SOC does not reliably indicate short-term management changes in carbon dynamics, the labile SOC pool serves as an early indicator of land-use changes. This labile pool, derived from decomposed materials such as plant residues and microbial biomass, is sensitive to early changes in carbon dynamics and soil quality. Active carbon (AC), or permanganate oxidizable carbon (POXC), is a vital part of the labile SOC pool, essential for supporting soil biology and regulating soil quality functions influenced by management practices (Weil et al., 2003; Stiles et al., 2011; Culman et al., 2012; Lucas and Weil, 2012).

Indeed, SOC was categorized into three types as active carbon (AC) with high microbial activity, intermediate carbon (IC) with lower activity, and passive carbon (PC) for long-term storage. IC acts as a bridge between AC and PC, displaying properties of both (Fang et al., 2012). Therefore, assessing the AC pool is more critical than evaluating the total SOC for understanding soil quality related to ecosystem services. Due to the high reactivity of activated carbon components, they can be readily destroyed by soil microbial communities (Bongiorno et al., 2019; Razzaghi and Celik, 2023). Microalgae, which are abundant and widely distributed throughout aquatic ecosystems, are photosynthetic microorganisms that synthesize metabolites with high organic matter content, including lipid-rich compounds, polysaccharides, nitrogenous compounds, and pigments, utilizing photosynthetically active radiation and mineral nutrients (Markou and Nerantzis, 2013; Karabulut, 2023). Although microalgae are rich in amino acids, they are also high in organic micronutrients, and their high vitamin B12 levels are noteworthy (Adsan and Eliçin, 2025). It is well established that microalgae play a crucial role in plant-microbial interactions within the rhizosphere, promoting root system development. Furthermore, certain microalgae genera exhibit biocontrol properties against plant pathogens, serving as biological control agents. They also support carbon sequestration by fixing atmospheric carbon dioxide (CO_2) through light-driven processes (Wang et al., 2015; Kılıç et al., 2025). Additionally, a previously conducted study reported that microalgae decompose plant and animal waste, exhibit antagonistic effects against phytopathogenic microorganisms, and are effective in plant nutrient cycling (Borowitzka, 1992, 1995; Demir, 2020). Due to the increased production of extracellular glycans by microalgae (Mahmood, 2016), there is an increase in the amount of organic carbon in the soil along with the increase in algal biomass in the soil (Mahmood, 2016; Kannaiyan et al., 1992). In addition, microalgae, along with fungi and certain rhizosphere bacteria, can sustain their lives in a mutualistic relationship with vascular plants, emerging as functional microbial fertilizers in ecological farming systems (Solomon et al., 2023; Okutan, 2024). When used as biofertilizers, algae are essential for plant root, stem, and leaf development, as well as increasing plant productivity, due to their content of trace elements and essential nutrients, certain plant hormones, polyamine group compounds, natural enzymatic compounds, carbon compounds, nitrogenous organic compounds, and organic micronutrients (Uysal et al., 2015; Doblan, 2024). In this regard, Bayram (2014) demonstrated that the application of microalgae along with organic fertilizer to melons improved various plant growth parameters, generally enhancing plant growth. *C. vulgaris* belongs to the Chlorophyceae family, which is rich in organic content, including lipids, nitrogenous compounds, micronutrients, and various other nutritional sources (Simanjuntak and Indarmawan, 2019; Wijaya and Prabaningtyas, 2024). *C. vulgaris* is a green microalgae species with a cellular nucleus, making it the most promising microalgae variety for biological applications. It is widely cultivated and used as a dietary supplement by individuals engaged in large-scale commercial production (Özdemir et al., 2016), as well as in the pharmaceutical and personal care sectors (Sharma et al., 2012). *C. vulgaris*, a type of microalga with a cellular nucleus, possesses advanced photosynthetic potential and exhibits a wide distribution in aquatic and terrestrial ecosystems of varying characteristics (İkiz and Daşgan, 2022). Microalgae, categorized as organic fertilizers in agriculture, are documented to enhance productivity and product quality (Norrie, 2008; Chojnacka et al., 2012; Acun and Bozokalfa, 2020). However, scientific research on their application across diverse cultivated plants remains relatively scarce (Silva et al., 2017; Acun and Bozokalfa, 2020). The primary aim of this study is to investigate, analyze, and assess the impact of *C. vulgaris*, a microalgal species, on the SOC fractions in the rhizosphere soils of onion (*Allium cepa*), garlic (*Allium sativum*), and leek (*Allium porrum* L.), which represent distinct vegetable species, thereby addressing a gap in the current literature.

2. Material and Methods

2.1. Experimental design and materials

The experiment was conducted for 60 days in 2025 in the Soil Science and Plant Nutrition Greenhouse of Erciyes University Faculty of Agriculture, Türkiye. The experiment was conducted using a Completely Randomized Design (CRD) with three replications per treatment for each plant species. The treatments consisted of *C. vulgaris* application and a control without microalgae application. For each *Allium* species, three replications were established under *C. vulgaris* treatment and three replications under control conditions. The plant material included leek (*Allium porrum* L.) 'Yerli Ata İnegöl', onion (*Allium cepa*) 'Kar Beyazı', and garlic (*Allium sativum*) 'Kastamonu/Taşköprü'. The *C. vulgaris* microalgae used in the experiment were obtained from the company Naturiga in powder form. The dose of *C. vulgaris* was determined based on the study by Dineshkumar et al. (2020) and calculated on a dry weight basis for the control (without microalgae) and the microalgae-applied (1 g kg^{-1}) treatments, and was homogeneously mixed into the soil of the pots. The pots for planting the seed were 3 liters in volume. The soil samples used in the experiment were collected from the 0-30 cm depth at the ERUTAM (Erciyes University Agricultural Research and Application Center) field (38°42'54"N 35°32'44"E) located in the Melikgazi district of Kayseri Province, Türkiye. The soil's physical and chemical properties were analyzed. The analyzed soils were added to 3 kg (3 liters in volume) plastic pots. Subsequently, 3 garlic cloves, 40 leek seeds, and 40 onion seeds were sown separately into pots. Leek and onion seedlings were thinned to 15 plants per pot after germination. All garlic cloves successfully emerged. The plants were watered daily with 200 ml of water per pot, ensuring uniform irrigation across treatments. Plant management practices were applied uniformly, and weeds were manually removed when necessary. At harvest, plant samples were collected and placed into labelled paper bags. Meanwhile, soil samples were collected from 0-15 cm depth and placed in labelled plastic bags.

2.2. Analysis Methods

2.2.1. Soil physical and chemical analysis

The soil was air-dried and then sieved through a 2 mm sieve. Soil texture analysis (Bauyoucos, 1954), pH and EC analysis (McLean, 1965), calcium carbonate ($CaCO_3$) content analysis (Allison and Moodie, 1965), and organic matter analysis (by the modified Walkley-Black procedure) (Walkley and Black, 1934) were performed to determine the soil's physical and chemical properties. According to the results obtained from soil analyses, the soil belonged to the sandy loam (SL) texture class, was slightly alkaline (pH 7.55), non-saline (ECe 0.792 dS m^{-1}), moderately calcareous (4.36 %), and had a low organic matter content (0.94 %) (Table 1).

Table 1. Physical and chemical properties of soils used in the experiment

Soil properties	**Results**
Texture	Loamy sand
pH (1:2.5)	7.55
EC (dS m^{-1})	0.792
$CaCO_3$ (%)	4.36
Soil organic matter (%)	0.94

2.2.2. Plant morphological characteristics

Plant samples were thoroughly washed in the laboratory with distilled water to remove dust and soil particles for post-harvest plant measurements. The height and root length of 15 plants of leek and onion, and all three plants from each harvested pot, were determined using a meter. The shoots and roots of the plants were weighed separately, and their fresh weights were determined using a precision balance. The plant samples were then placed in paper bags and dried in an oven at 70 °C until constant weight. Samples brought to a constant weight were removed from the oven, and their dry weights were determined using a precision balance (Kacar and İnal, 2008).

2.2.3. SOC fractions

To determine AC, a 5 g sample of air-dried soil was placed in a 50 ml screw-top polypropylene tube and shaken with 25 ml of 0.02 M $KMnO_4$ (buffered at pH 6) at 100 rpm for 2 minutes using a reciprocal shaker. Following shaking, the soil suspensions underwent centrifugation at 3000 rpm for 5 minutes to yield soil-free aliquots. A 0.5 mL aliquot was diluted to a final volume of 50 mL, and the absorbance of the AC concentration was measured spectrophotometrically at 550 nm using a series of standards for comparison, subsequently, IC and PC were calculated (Blair et al., 1995; Islam et al., 2021; Razzaghi et al., 2022; Razzaghi, 2025).

2.3. Statistical analysis

Statistical analyses and graphical representations were performed using SPSS and Microsoft Excel software. For plant parameters, three replicates were used per treatment. For soil analyses, three replicates were taken, with each replicate consisting of two subsamples. Differences between the algae treatment and the control were evaluated using an independent samples t-test. Statistical significance was determined at the 5% and 1% significance levels ($p<0.05$ and $p<0.01$, respectively).

3. Results

3.1. The morphological traits

The variance of *C. vulgaris* treated and control treatments was not statistically significant for all studied morphological traits of leek plants (Table 2). As mentioned earlier, even though the differences were not statistically significant, the leek plants treated with *C. vulgaris* generally had higher mean values for all morphological traits, including plant fresh weight (17.79 g), plant dry weight (1.85 g), plant height (37.3 cm), root length (7.7 cm), root fresh weight (2.31 g), and root dry weight (0.33 g) compared to the control (Table 3). This suggested that a positive trend may emerge and become significant under different environmental or management conditions.

Table 2. Effects of *C. vulgaris* application on morphological traits of leek (*Allium porrum* L.) (T-Test)

	Plant fresh weight (g)	**Plant dry weight** (g)	**Plant height** (cm)	**Root length** (cm)	**Root fresh weight** (g)	**Root dry weight** (g)
t Value	1.44	1.29	1.47	0.42	0.66	0.009
df	4	4	4	4	4	4
Probability	0.22 ns	0.26 ns	0.21 ns	0.70 ns	0.54 ns	0.99 ns

ns = Indicates a non-significant difference between measured plant morphological characteristics under treatments at p≤0.05.

Table 3. The morphological traits at 0–15 cm soil depth in *C. vulgaris* treated and control leek, onion, and garlic plants

Plants		**Means**					
		Plant fresh weight (g)	**Plant dry weight** (g)	**Plant height** (cm)	**Root length** (cm)	**Root fresh weight** (g)	**Root dry weight** (g)
Leek	*C. vulgaris*	17.79 ± 3.79 a	1.85 ± 0.37a	37.3 ± 2.3 a	7.7 ± 0.8 a	2.31 ± 0.63 a	0.33 ± 0.08 a
	Control	11.62 ± 6.36 a	1.27 ± 0.67a	32.3 ± 5.1 a	7.4 ± 1.2 a	1.81 ± 1.14 a	0.32 ± 0.16 a
Onion	*C. vulgaris*	3.007 ± 1.42 b	0.23 ± 0.12 b	25.50 ± 2.50 a	6.7 ± 4.2 a	0.38 ± 0.19 a	0.05 ± 0.006 b
	Control	11.71 ± 3.13 a	1.15 ± 0.39 a	25.50 ± 0.50 a	7.5 ± 0.9 a	1.81 ± 0.98 a	0.25 ± 0.11 a
Garlic	*C. vulgaris*	3.29 ± 1.94 a	1.58 ± 0.89 a	38.61 ± 9.93 a	9.4 ± 0.7 a	6.20 ± 4.10 a	5.9 ± 3.36 a
	Control	4.30 ± 1.55 a	1.55 ± 0.30 a	44.13 ± 8.52 a	7.7 ± 0.2 b	7.60 ± 1.68 a	5.50 ± 1.61a

[*] Means within each column that share a similar letter(s) are not significantly different at a 5% probability level

The highly significant difference in onion fresh weight (t=4.38; p=0.0119), dry weight (t=3.87; p=0.02), and root dry weight (t=3.05; p=0.038) in the t-test values indicated that *C. vulgaris* treatment enhanced biomass accumulation compared to the control group. Furthermore, no statistically significant variations were observed in plant height (p=1), root depth (p=0.78), and root fresh weight (p=0.07) (Table 4). The fresh and dry weights in the control treatment for onions were 11.71 g and 1.15 g, which were interestingly higher than those of onions treated with *C. vulgaris*, at 3.007 g and 0.23 g, respectively. Similarly, the root dry weight with 0.25 g was higher than that of onions roots treated with *C. vulgaris* with 0.05 g. As mentioned above, although there was no statistical significance, the other morphological treatments in the control were higher than those with the *C. vulgaris* treatment (Table 3).

Table 4. Effects of *C. vulgaris* application on morphological traits of onion (*Allium cepa*) (T-Test)

	Plant fresh weight (g)	**Plant dry weight** (g)	**Plant height** (cm)	**Root length** (cm)	**Root fresh weight** (g)	**Root dry weight** (g)
t Value	4.38	3.87	0.00	0.30	2.49	3.05
df	4	4	4	4	4	4
Probability	0.0119*	0.02*	1 ns	0.78 ns	0.07 ns	0.038*

ns and * = indicate a non-significant and significant difference between measured plant morphological characteristics under treatments at p≤0.05, respectively.

The *C. vulgaris* application had a statistically significant effect on garlic root length (p≤0.05), indicating deeper root growth into the soil profile (Table 5). There were no significant differences in the plant fresh and dry weights, plant height, or root fresh and dry weights of the garlic plants. Although differences in the studied morphological characteristics were not statistically significant, except for root length, the other parameters showed higher levels in the control group than in *C. vulgaris*-treated plants (Table 3). In addition, the root length of garlic treated with *C. vulgaris* (9.4 cm) was much longer than that of the control (7.7 cm) (Table 3).

Table 5. Effects of *C. vulgaris* application on morphological traits of garlic (*Allium sativum*) (T-Test)

	Plant fresh weight (g)	**Plant dry weight** (g)	**Plant height** (cm)	**Root length** (cm)	**Root fresh weight** (g)	**Root dry weight** (g)
t Value	0.70	0.06	0.73	3.82	0.55	0.65
df	4	4	4	4	4	4
Probability	0.52 ns	0.95ns	0.51 ns	0.0187*	0.61 ns	0.55 ns

ns and * = indicate a non-significant and significant difference between measured plant morphological characteristics under treatments at p≤0.05, respectively.

3.2. SOC fractions

Under *C. vulgaris* treatment, no significant response was observed (p≥0.05) in some SOC fractions like AC, IC, and LC. On the other hand, PC and total SOC increased significantly in the PC and total SOC groups compared to the control group (p≤0.05) (Table 6). These findings suggested that *C. vulgaris* augmented the more stable and enduring SOC pools rather than the swiftly cycling carbon fractions in leek soils. *C. vulgaris* significantly increased the amount of passive carbon (PC) in leek soil by 37%, from 4.60 to 6.31 g kg^{-1}. Similarly, the total SOC increased by 33.7% when *C. vulgaris* was used (Figure 1). As mentioned above, despite no significant difference, AC increased by only 0.54%, while labile C remained relatively unchanged under *C. vulgaris* compared with the control. Under *C. vulgaris* treatment, IC decreased by 10% compared with the control (Table 7).

Table 6. Effects of *C. vulgaris* application on soil organic carbon (SOC) Fractions (active carbon (AC), passive carbon (PC), intermediate carbon (IC), and labile C (LC)) and SOC concentrations of leek (*Allium porrum* L.) (T-Test)

	AC (mg kg^{-1})	**IC** (mg kg^{-1})	**PC** (g kg^{-1})	**LC** (mg kg^{-1})	**SOC** (g kg^{-1})
t Value	1.26	1.60	2.46	1.13	2.48
df	5	5	10	10	10
Probability	0.23 ns	0.14 ns	0.03*	0.28 ns	0.03*

ns and * = indicate a non-significant and significant difference between measured plant morphological characteristics under treatments at p≤0.05.

Table 7. Active carbon (AC), passive carbon (PC), intermediate carbon (IC), labile C (LC), and soil organic carbon (SOC) concentrations at 0–15 cm soil depth in *C. vulgaris* treated and control leek, onion, and garlic plants

Plants		**Means**				
		AC (mg kg^{-1})	**IC** (mg kg^{-1})	**PC** (g kg^{-1})	**LC** (mg kg^{-1})	**SOC** (g kg^{-1})
Leek	*C. vulgaris*	608.44 ± 1.85 a*	35.00 ± 0.77 b	6.31 ± 1.34 a	643.45 ± 1.17 a	7.02 ± 1.4 a
	Control	605.17 ± 6.07 b	39.02 ± 6.07 a	4.60 ± 1.03 b	644.20 ± 1.12 a	5.25 ± 1.0 b
Onion	*C. vulgaris*	616 ± 3.01 a	43.34 ± 2.91 b	5.99 ± 0.50 a	659.50 ± 5.17 a	6.80 ± 0.7 a
	Control	607.41 ± 1.42 b	52.13 ± 2.01 a	4.64 ± 0.79 b	659.55 ± 3.29 a	5.30 ± 0.79 b
Garlic	*C. vulgaris*	609.94 ± 0.93 a	59.39 ± 0.59 b	6.13 ± 1.10 a	669.33 ± 0.54 a	6.8 ± 1.10 a
	Control	608.72 ± 1.97 a	60.46 ± 0.66 a	4.58 ± 0.74 b	669.19 ± 1.48 a	5.25 ± 0.7 b

*= Means within each column that share a similar letter(s) are not significantly different at a 5 % probability level

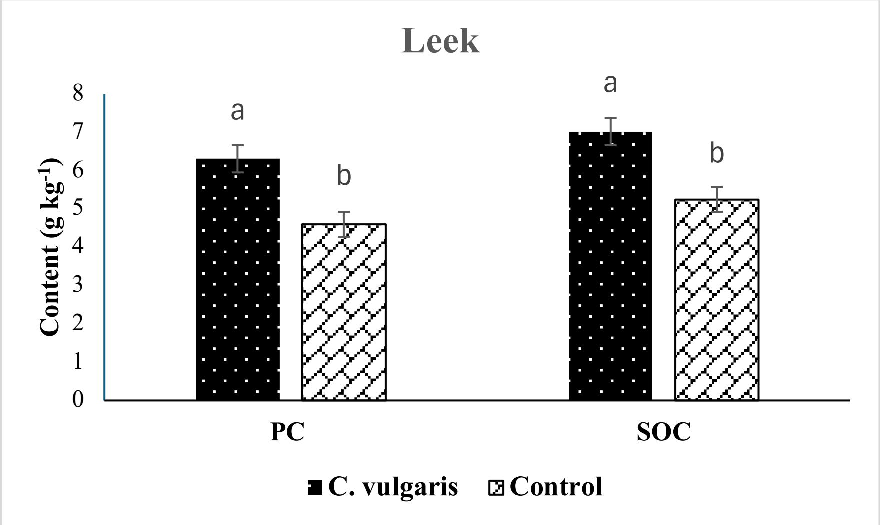

Figure 1. The soil PC and SOC contents with *C. vulgaris* treatment and the control in the leek plant

The onion reacted the most to the *C. vulgaris* treatment among the other studied plants. The soil AC, IC, PC, and SOC contents were significantly different under the studied treatment (p≤0.05) (Table 8). The soil AC content was higher in the *C. vulgaris*-treated soil compared to the control. The application of *chlorella* increased soil AC, PC, and SOC by 1.4%, 29%, and 28%, respectively (Table 8). Compared to the control, the soil IC decreased by 17%, while the LC content remained approximately the same (≈659.5). These results indicate that when *C. vulgaris* was added to onions, both the active and stable carbon pools showed significant improvement. The increase in PC and SOC content in *C. vulgaris*-treated soil compared to the control is exhibited in Figure 2.

Table 8. Effects of *C. vulgaris* application on soil organic carbon (SOC) Fractions (active carbon (AC), passive carbon (PC), intermediate carbon (IC), and labile C (LC)) and SOC concentrations of onion (*Allium cepa*) (T-Test)

	AC (mg kg^{-1})	**IC** (mg kg^{-1})	**PC** (g kg^{-1})	**LC** (mg kg^{-1})	**SOC** (g kg^{-1})
t Value	6.44	6.09	3.54	0.019	3.48
df	10	10	10	10	10
Probability	0.0001**	0.0001**	0.0054**	0.98ns	0.0059**

ns, * and ** = indicate a non-significant and significant difference between measured plant morphological characteristics under treatments at p≤0.05 and p≤0.01, respectively.

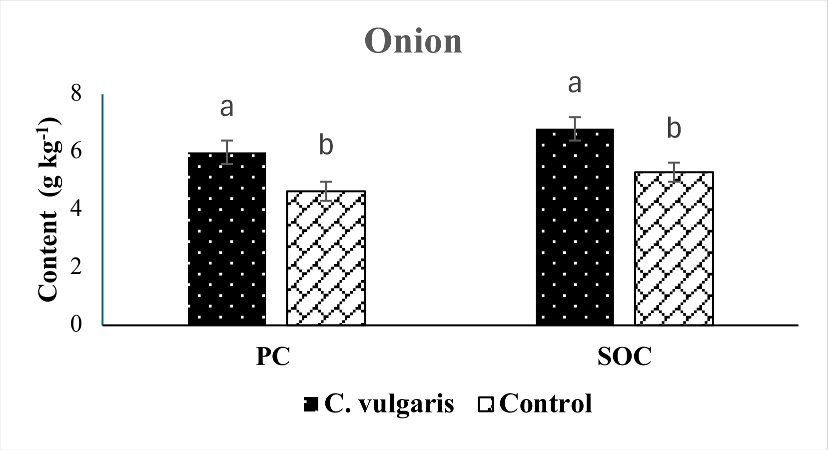

Figure 2. The soil PC and SOC contents with *C. vulgaris* treatment and the control in the onion plant

Garlic soils showed a bigger reaction to *C. vulgaris* than leek soils did. IC, PC, and SOC were significantly different across the studied treatments (p≤0.05). However, there was no statistically significant variation in soil AC and LC content under measured treatments (Table 9). Compared with untreated soil, PC increased by approximately 34% and SOC increased by 29% under *C. vulgaris* treatment (Figure 3). Although there were no significant differences in AC and LC content under the *C. vulgaris*-treated soil, the concentrations of AC and LC were 0.2% and 0.02% higher, respectively, than in the control treatment. IC content decreased slightly (−1.7%) under *C. vulgaris* treatment compared with the control (Table 7).

Table 9. Effects of *C. vulgaris* application on soil organic carbon (SOC) Fractions (active carbon (AC), passive carbon (PC), intermediate carbon (IC), and labile C (LC)) and SOC concentrations of garlic (*Allium sativum*) (T-Test)

	AC (mg kg^{-1})	**IC** (mg kg^{-1})	**PC** (g kg^{-1})	**Labile C** (mg kg^{-1})	**SOC** (mg kg^{-1})
t Value	1.37	3.00	2.85	0.22	2.86
df	10	10	10	6	10
Probability	0.20 ns	0.013*	0.017*	0.83 ns	0.017*

ns and * = indicate a non-significant and significant difference between measured plant morphological characteristics under treatments at p≤0.05, respectively.

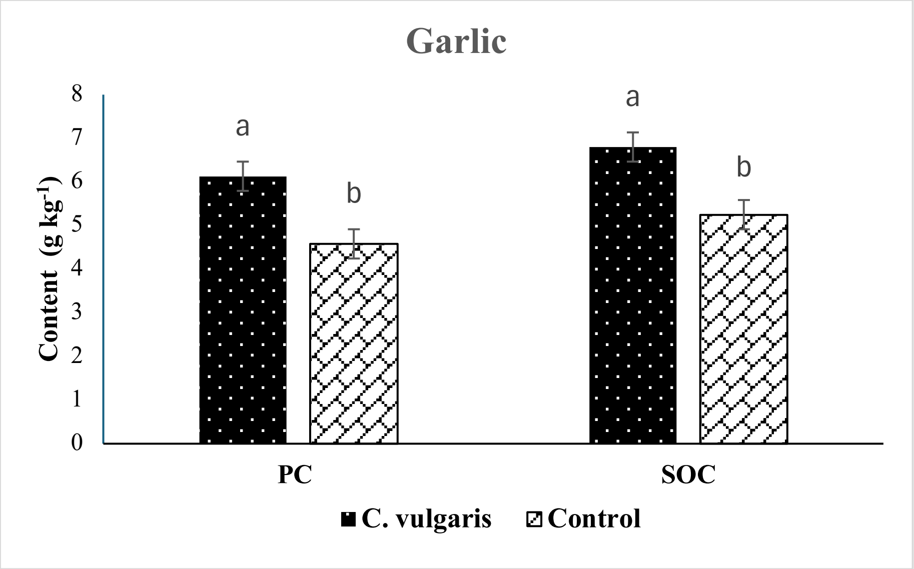

Figure 3. The soil PC and SOC contents with *C. vulgaris* treatment and the control in the garlic plant

4. Discussion

4.1. The morphological traits

The *C. vulgaris* applications played a significant role in the growth of *Allium* crops, with different responses observed in leek, garlic, and onion. These results indicate that *C. vulgaris* is influenced by the type of plant and the environmental conditions in which it grows (Kılıç et al., 2025). In leek plants, despite the lack of statistically significant differences in morphological traits, due to slower root turnover and lower metabolic plasticity than in garlic and onion, *C. vulgaris* generally had higher mean values for all morphological traits than the control. According to Sil Kim et al. (2019), *Chlorella* application enhanced leek performance by decreasing gray mold disease incidence by 22.5% and augmenting fresh weight by 9.0% relative to untreated controls, demonstrating its efficacy as a growth-promoting and disease-suppressing biostimulant in leek cultivation. Furthermore, Senousy et al. (2023) reported that the application of algal extracts from *C. vulgaris* and *Dunaliella salina* enhanced morphological traits, such as biomass accumulation and growth, in *Phaseolus vulgaris* (common bean) plants under salinity stress. Unlike several previous studies, which suggested that *C. vulgaris* and microalgae enhanced chlorophyll concentrations and photosynthesis-related characteristics, resulting in increased growth, the morphological characteristics of wheat, maize, bean, lettuce, and onion plants (Fattah, 2008; Schreiber et al., 2018; Dineshkumar et al., 2019; Faheed and El-Gemin et al., 2019; Mutale-Joan et al., 2021; Gharib et al., 2024). The control onion plants and roots exhibited higher fresh and dry weight, possibly due to adequate nutrient availability and optimal growth conditions, which diminished the impact of *C. vulgaris* application. The biostimulant effect of *C. vulgaris* in the absence of abiotic stress may be constrained, resulting in fresh weight comparable to or lower than that of control plants. In the garlic plant, *C. vulgaris* promoted root elongation, which enhanced soil penetration due to the production of auxin-like, cytokinin-like compounds, vitamins, and polysaccharides by microalgae, which affect cell division and elongation in roots (Ördög et al., 2004; Singh, 2014; Ferreira et al., 2023; Razzaghi, 2024b; Gharib et al., 2024). Al-Esaily and Ismail (2010) found that applying a green microalgae extract (*Scenedesmus* sp.) to garlic leaves improved plant growth, increased mineral uptake, increased chlorophyll content, and increased fruit production, especially in sandy soil. Moreover, extracts from microalgae, particularly *C. vulgaris* and *Scenedesmus quadricauda,* have been shown to enhance root elongation, fine root development, and the number of root tips in sugar beet seedlings, thereby promoting nutrient absorption (Barone et al., 2018). The reaction to *C. vulgaris* differed among the three *Allium* species: leek showed a mild, non-significant response, garlic showed a substantial increase in root length, and onion predominantly increased fresh biomass, particularly in the control. This signifies a plant species-specific impact of microalgal interventions.

4.2. SOC fractions

According to the results, all crops exhibited higher AC, PC, and SOC content in the *C. vulgaris*-treated soils than in the control. However, the statistical significance and the increase ratio compared to the control varied. Total SOC increased significantly across all crops (≈28–34%), highlighting that *C. vulgaris* boosted soil carbon stocks. Similar findings by Swami et al. (2025), indicated that edaphic microalgae, such as *C. vulgaris*, markedly augment SOC by photosynthetic carbon fixation, biomass accumulation, extracellular polymeric substances (EPS) formation, carbon stabilization, and soil aggregation, resulting in SOC increases of 15–30% depending on soil type and management approaches. Gougoulias et al. (2018) also reported that the input and biomass of *C. vulgaris* enhanced the mineralization of soil organic matter (SOM) and nutrient availability, without adversely affecting soil chemical characteristics. The microbial biomass from algae enhances organic matter accumulation and fosters humification through microbial transformation. In this line, Abd El-Tawwab et al. (2025) reported that compost-biochar additions and *C. vulgaris* extract can boost SOM and microbial activity. They suggested potential indirect advantages for SOC dynamics, hence improving biomass production and microbial turnover. Similar results have been reported by previous studies (Zhang et al., 2018; Zaimenko et al., 2023). PC is connected to mineral-bound organic matter, which decomposes over a long period and can improve soil fertility (Islam and Weil, 1998; Islam et al., 2021). The rise of PC contents (≈29–37 %) in all crops indicated the special role of the microalgae *C. vulgaris* in enhancing the stable and long-term fraction of SOC, consistent with the findings of Zhou et al. (2023) who reported that *C. vulgaris* can enhance decomposition of SOM and increase SOC. This phenomena also improve soil health even under abiotic stress conditions (Maurya et al., 2025). Consistently, *C. vulgaris* has demonstrated the ability to enhance rice growth by producing compounds like auxins, cytokinins, and gibberellins, while simultaneously improving the soil microecosystem, augmenting soil fertility and health (Chanda et al., 2019). IC, which possesses characteristics of both AC and PC segments, functions as a conduit between the two (Fang et al., 2012; Razzaghi, 2025). The significant reduction in IC with *C. vulgaris* inputs in all crops may indicate a rapid conversion to stable components, corresponding with rises in PC and SOC. The AC and labile portions of SOC undergo rapid turnover in soil (Rovira et al., 2010; Razzaghi et al., 2022; Rakesh et al., 2023). In leek and onion plants, soil AC contents were significantly affected by *C. vulgaris* inputs, whereas in garlic plants, this rapid turnover fraction of the soil was not significant. The statistical significance of soil AC did not imply that this fraction may vary with *C. vulgaris* treatment, as the AC and LC content of the soil in all studied crops exhibited negligible variation (0–1%) from *C. vulgaris* inputs, suggesting that these inputs did not significantly influence fast-cycling carbon reservoirs. This outcome suggests that microalgal inputs favor carbon stabilization over short-term mineralization, indicating a transition toward improved carbon retention and long-term stability and, thereby, as indicated above, enhancing soil health and crop productivity (Razzaghi, 2022b; Razzaghi, 2024b). Among the assessed crops, the Onion showed the most response to *C. vulgaris* application. According to the results, after onion and garlic plants, leek plants exhibited a reaction to SOC and its fraction when treated with *C. vulgaris*. This suggested that rhizosphere conditions differ among crops and may influence the stabilization of *C. vulgaris*-derived carbon, which is shaped by plant species and root exudate composition (Razzaghi, 2024b; Razzaghi, 2025). Overall, the results indicated that the application of *C. vulgaris* increased soil SOC and PC content, while the LC and AC fractions decreased. Along with the positive impact of *C. vulgaris* on soil chemical properties, as indicated previously, soil physical properties, such as soil structure and aggregation, improved, thereby leading to a decrease in the labile fraction (AC) and an increase in PC and SOC. This result is consistent with Mason et al. (2023), who reported that soil aggregates inhibit the decomposition of soil organic carbon (SOC) by facilitating the transfer of carbon from labile to recalcitrant pools.

5. Conclusion

The findings of this study indicated that the application of *C. vulgaris* had minimal immediate impact on the morphology of leek, onion, and garlic plants. Most growth measures exhibited no statistically significant changes among treatments. Nonetheless, favorable changes were observed in leeks, and a notable increase in root length was observed in garlic. The results suggested that *C. vulgaris*'s impact on plant growth may differ by crop and be more substantial in root system development than in aboveground biomass, perhaps intensifying under diverse climatic circumstances or prolonged trial durations. The persistent and significant use of *C. vulgaris* consistently increased SOC levels across all studied crops. The passive and total SOC fractions showed the greatest changes, suggesting that *C. vulgaris* contributes to the long-term stabilization and sequestration of carbon in soil rather than accelerating the carbon cycle. Onion and garlic soils demonstrate notably significant effects, suggesting that *C. vulgaris* and soil carbon processes interact differently across crop types. The research indicated that *C. vulgaris* is superior as a soil conditioner, improving carbon sequestration and soil quality, rather than serving as a temporary stimulant for plant development, hence endorsing its prospective application in sustainable agriculture and soil management strategies.

Declaration of Author Contributions

All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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Ethical Committee Approval

This study did not involve human or animal subjects; therefore, ethical approval was not required.

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