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Research Article

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The effect of mycorrhiza-inoculated succession-planted main crop (maize-wheat) and cover crop rotation on soil organic carbon

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ABSTRACT
There has long been intense pressure on input-based traditional agriculture due to the rising need for food. These kinds of agricultural techniques result in poorly managed soil-plant interactions that ultimately affect all living activities and human health by altering and destroying the soil's natural structure, rendering it infertile, and degrading soil quality. Research was carried out a study to find out how mycorrhizae-inoculated cover crops affected soil quality and growth metrics. The study to pot experiment was carried out under greenhouse conditions. Five different plant combinations (cover crop) patterns were inoculated with selected and indigenous mycorrhizae spores that were isolated from the rhizosphere soils of plants grown in three degraded soils. Five different cover plant combinations, such as A: Clover, Grass, Onion; B: Faba Bean, Grass, Safflower; C: Clover, Grass, Safflower, Faba Bean, Onion; D: Maize; and E: Wheat, were planted in 7 kg soil-containing pots with three replications. The seeds of different plants were planted per m2 surface of each pot, with the number of seeds per pot calculated depending on their sowing amount per hectare. Sixty days after planting, the plants were harvested by cutting 0.5 cm above the soil surface with scissors. In the second rotation experiment, after the harvest of the first rotation experiment, maize (*Zea mays* L.) was planted on the A, B, C, and D patterns, and wheat was planted on the E pattern. In the third rotation, wheat was sown on all pots after the second maize harvest. As a result of successive three-pot experiments on the same soils, were analyzed for the soil organic carbon (SOC) concentrations. In general, treatments with Funneliformis mosseae followed by indigenous mycorrhiza spores led to an increase in soil OC concentration compared to control treatments. SOC concentrations in Havutlu and Arık soils were higher than those in Avadan soil. Avadan is a highly eroded soil and has high lime content, high pH, and low soil fertility. When consecutively planted trials are examined, the soil SOC concentration increased from the first trial to the last trial for all soils. The obtained SOC results seem to partially support the research hypothesis. The results revealed that poorly managed soil requires rehabilitations with various combinations of cover crops.
Keywords: Cover crops, Mycorrhizal inoculation, Wheat, Maize, Soil organic matter

INTRODUCTION
Soil is one of the most complex systems in the world due to its physical, chemical, and biological properties. Soils are the only source that provides 98.8% of our food. However, beyond expectations, agricultural soils, which constitute a very small part of the terrestrial ecosystem in the world, function as a living element despite being a limited resource (Ortas et al., 2021; Ortaş, 2017). While the human population increased from 250 million in 1000 to 6.1 billion in 2000, it is predicted to increase to 9.8 billion by 2050 (Kopittke et al., 2019). World population growth, which has doubled every 50 years in the last century, creates an increasing demand for food production on land. Currently, 42% of the world's population makes their living by farming, and agriculture determines the economies of many developing countries. For this reason, human life on our planet depends on sustainable agriculture (Aznar-Sanchez et al., 2019). Assumptions from the world's population show that in the 2050s, to meet food needs, demand will increase by 70% compared to the levels needed for human nutrition today, (Krishnan et al., 2020). With the increase in population, the growing need for agricultural products has been addressed by increasing the yield with intensive chemical fertilizers and pesticides. Unfortunately, the negative effects of excessive use of chemical inputs in agricultural activities, on human and environmental health, have accumulated over time. The increasing demand and consumption of food lead to the intensification of agricultural production and increased soil inputs to boost the soil per unit area, resulting in the rapid deterioration of soil quality and a decrease in its productivity.

As a result, sustainable agricultural systems that are based not only on increasing yields but also on protecting human health and the environment have gained importance (Eryılmaz et al., 2019). In areas where agriculture has been carried out for years, inappropriate heavy soil tillage management, excessive chemical use, and monoculture have negatively affected the natural vitality of the soil. In agricultural areas where continuous monoculture is carried out, the unilateral decrease in certain mineral nutrients from the soil over time, the differentiation of certain microorganism communities (Börner and Özer, 2010) and especially the negative effects on organisms such as mycorrhizal fungi, and deterioration of the soil biological activity of the soil fatigue (Politycka, 2005). As expected, soil fatigue and poor soil quality reduce soil productivity or yield. To bring the soil back to a productive state, a series of special measures such as plant rotation, organic matter addition, and/or microorganism inoculations has been tried. Mycorrhizal fungi establish a symbiotic relationship with some plant roots (Compant et al., 2010; Schwartz et al., 2006) and provide nutrients (Harrison and van Buuren, 1995), while the plant provides carbohydrates (Javaid, 2009) for its energy supply (Smith and Read, 2010). Also, mycorrhiza protects the plants from stress factors and plays a unique role in the healing and balanced nutrition of soils. In this context, mycorrhizal fungi act as a bridge between the root and the soil and play an active role in the transportation of nutrients from the soil to the plant roots. Physiological and morphological changes occur in the plant roots with mycorrhizal inoculation; some events, such as competition, contribute to plant development (Yildiz, 2009).

In this context, various ecological practices or suggestions have been presented in recent years. For example, it is emphasized that many suggestions such as minimum tillage, crop rotation, cover crops and sensitive water/nutrient management have positive effects on soil fertility, increase soil biodiversity and improve carbon sequestration and nutrient storage, which directly influence sustainability (Jat et al., 2022). These mechanisms are necessary for sustainable agriculture.

The application of cover crops, to mixed cropping areas, and the natural mycorrhizal fungi in the soil, both of which are ancient natural mechanisms, are extremely important for sustainability. The application of these practices can restore the biological fertility of the soil. Cover crops and mycorrhizal fungi (MF) to can stimulate the recovery of degraded agricultural soil structure, soil quality can be improved, and the sustainability of the agroecosystem can be restored. Despite all this information, the effects of cover crops and their interactions with mycorrhizal fungal communities and the mechanisms of their adaptation to soil ecological conditions are still not very well known (Hontoria et al., 2019).

As a result of the use of the natural mechanisms of plants in soil improvement, understanding the potential of different plants to capture carbon in the atmosphere and release carbon in the subsoil is important. In addition, the storage of carbon released to the underground root zone is also an important research topic. The increase in the carbon stock, especially organic carbon, in the soil improves the quality and maintains the soil biological productivity. At the same time, the carbon stock contributes to decreasing atmospheric CO_2 concentrations. Ortas and Yucel (2020) reported that mycorrhizae inoculation significantly increased cover crop growth and mycorrhiza stimulated plant carbon fixation. In this way, sustainable agriculture mitigates climate change effects. In addition to selecting ideal cover crops suitable for each region according to land use, the accumulation of organic matter in the soil will improve the physical conditions and increase its biological richness, while also enhancing the chemical productivity and quality of the soil (Şimşekli and Kapur, 2012)

As a result of the increasing demand for food along with the increasing human population, This research is aimed to provide rehabilitation of soils by inoculating cover crops with mycorrhizae spores in three different types of soils where traditional agriculture with intensive input has been practised for many years; poor soil-plant management has been applied.

MATERIALS AND METHODS

Site and Experimental Descriptions

Experiments were established in three different poorly managed soils, with the 2-factor randomized plots experimental design in the Research and Application Greenhouses of the Soil Science and Plant Nutrition Department of the Faculty of Agriculture of Cukurova University.

The soil, mycorrhiza and plant types used in the experiment are as follows.

S1: Lands where long-term traditional agriculture is done (Havutlu, Yuregir/Adana) (Wheat-maize cultivation is done with a rotation system)

S2: Long-term continuous phosphorus application (Arık series, under 200 kg P_2O_5 ha^{-1} application in the University campus of Cukurova)

S3: Water-eroded soil unsuitable for agriculture (Avadan soil on the side of Tarsus/Mersin)

In terms of mycorrhiza, three different mycorrhizal inoculations were performed.

M1: Without Mycorrhizal inoculation

M2: *Funneliformis mosseae*: It is propagated in an alfalfa host plant medium. According to the spore count measurements in the medium before the trial, the number of Fu. mosseae spores was measured as 97 spores/10g medium.

M3: Indigenous mycorrhizae: Spores isolated from the rhizosphere soils of the plants, in the area, where all three mismanaged soils were collected, were obtained as a result of the multiplication of the host plants using trap culture management. The indigenous mycorrhiza counts of all three soils before the experiment are given in Table 1.

Five different plant patterns were planted on the inoculated soils, 3 plant patterns were cover plants, and the remaining 2 plant patterns were selected as regular rotation plants such as maize and wheat.

Cover crops and mixtures of these cover crops used in the experiments are such as;

Faba Beans (Luz de otono variety)
Alexandrian Clover
Italian Grass
Safflower
Onion
Maize (Kebeos maize variety)
Wheat (Adana 99 variety)

Grouping of cover crop patterns

A Plant pattern: Alexandrian clover, Italian grass, Onion.
B plant pattern: Faba Bean, Italian grass, Safflower.
C plant pattern: Alexandrian clover, Italian grass, Onion, Faba Bean, Safflower)
D plant pattern: Maize
Plant pattern E: Wheat

Some physical and chemical properties of the soils used in the experiment are given in Table 1.

Experimental Setup Description

Sterilized soil was filled into 7 L capacity pots and 3 different cover crops (A, B and C) were used as the D group plant along with maize (*Zea mays L.)* and wheat (*Triticum aestivum*) seeds were planted as group E. A total of 3 rotations were set up and conducted on the same soil-filled pots in succession. The experiment consisted of 135 pots with three replications. The trial design is shown in Figure 1.

In the first rotation trial, five different plant patterns were placed in the pots: A: Alexandrian clover, Italian grass, Onion; B: Faba Bean, Italian grass, Safflower; C: Alexandrian clover, Italian grass, Safflower, Faba Bean, Onion; D: Maize; and E: Wheat. Seeds of different plants corresponding to these patterns were planted.

In the second rotation trial, after the harvest of the first rotation trial, maize seeds were planted in the A, B, C, and D patterns in the same pots of five different plant patterns, and wheat seeds were planted again in the E group pots.

In the third rotation trial, only wheat seeds were planted in all pots after the maize and wheat plants were harvested.

Table 1. Some physical and chemical properties of the experimental soils.

Soil	Texture	Lime	Total N	Total C	OC	IC	K	Zn	F	Mn	Cu	Indigenous M. Spores
		%					Mg kg -1					Spore/10g soil.
T1 (Havutlu)	Clay Loam	15.31	0.15	-	-	1.84	184.63	0.27	3.97	2.79	1.74	95
T2 (Arik)	Clay Loam	25.55	0.15	4.05	0.98	3.07	255.25	1.24	2.22	3.99	0.98	76
T3 (Avadan)	Silty Loam	46.03	0.13	5.59	0.07	5.52	53.38	0.15	0.73	0.75	0.31	46

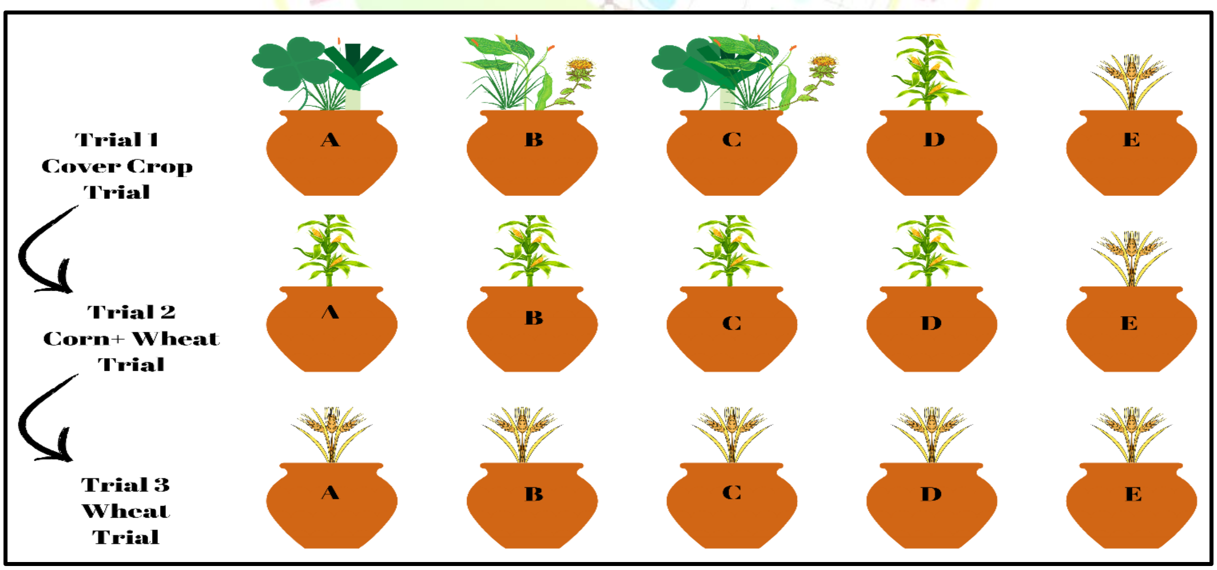

Figure 1. Rotation design of the experimental plots.

Harvesting Trials and Preparation for Analysis

A total of 1215 sample analyses were performed on plant and soil materials in 405 pots during the experiment, which consisted of 135 pots set up and finished in three stages in rotation. Within the scope of the research, 3 rotation trials were carried out, and these trials were planted and harvested.

In the first rotation trial, the cover crop was established on April 2, 2021, and harvested on May 23, 2021. The illustration of the trial is given in Figure 1.

In the second rotation trial, maize and wheat seeds were planted on June 11, 2021, and harvested on August 10, 2021. The rotation design of the trial is given in Figure 1. In the third and final rotation trial, the wheat seed planting was established on October 7, 2021 and harvested on December 14, 2021. The rotation design of the trial is given in Figure 1.

Chemical and Biochemical Properties of Soil

Total C (TC) were determined in oven-dried and <125-μm diameter fine soils using the CNS analyzer (Thermo-Fisher - 2000) according to the Dumas dry combustion method. To obtain soil organic carbon data, $CaCO_3$ was determined using a digital calcimeter, and inorganic carbon (IC) was obtained by subtracting $CaCO_3$ from total inorganic carbon.

Statistical Analysis and Interpretation

After the root, root surface, and rhizosphere soil samples of three rotation trials were analyzed and evaluated, the data for each rotation was recorded separately in the Excel data sheet. For the results of each experiment, ANOVA was performed using the JMP package program. The smallest differences between the applications were determined by the Tukey test.

RESULTS AND DISCUSSION

Soil Organic Carbon

Carbon that can be found in organic materials is known as soil organic carbon (SOC). Microorganisms, plant remnants, animal waste, and the byproducts of organic matter breakdown make up SOC. SOC is vital for soil health and fertility and contributes to the transformation and regulation of the atmosphere by participating in the carbon cycle. Under climate change conditions, keeping more soil organic carbon in the soil pool is vital for sustabanile soil management.

TOC is one of the parameters affecting soil fertility and helps provide the nutrients that plants need. It increases the ability of soils to retain and manage water, increases the aggregate stability of the soil, provides resistance to erosion, and contributes to the storage of carbon by withdrawing it from the atmosphere in the fight against climate change. Sustainable agricultural methods, organic fertilizers, cover crops, and correct soil management are critical to increasing soil organic carbon.

In this study, the effects of three different soils, three different mycorrhizae, and five different plant patterns on soil organic carbon content were investigated. The effects of three consecutive planting trials for each soil were considered separately. Data obtained for Havutlu soil 1 are presented in Figure 2, data obtained for Arık soil 2 are presented in Figure 3, and data obtained for Avadan soil 3 are presented in Figure 4. It appears that the prior rotation had an impact on the subsequent rotation, as three consecutive experiments were carried out after one another. This is crucial for the physical and biological fertility of soil as well as its overall health.

When the effects of successive planting trials on mycorrhizas inoculated into Havutlu soil (Figure 3.) soil organic carbon content was examined, the differences between the means in the mycorrhiza factor in the 2nd trial ($P<0.0448^*$) and in both the mycorrhiza ($P<0.0012^{**}$) and plant factor ($P<0.0001^{***}$) in the 3rd trial were found to be statistically significant.

When the TOC content was examined after the cover crop trial (Trial 1), the highest values were obtained as 1.7% for the interaction in the M3*E application, 1.62% for mycorrhiza in the M3 application and 1.62% for the plant pattern in the E plant pattern. When TOC content was examined after the maize+wheat trial (2nd Trial), the highest values were obtained as 1.79% for interaction in M3*D application, 1.63% for mycorrhiza in M3 application and 1.67% for plant pattern in D plant pattern. When TOC content was examined after the wheat trial (3rd Trial), the highest values were obtained as 2.48% for interaction in M2*E application, 2.21% for mycorrhiza in M2 application and 2.31% for plant pattern in E plant pattern. (Figure 2.)

When the effects of the trials planted consecutively on mycorrhizas inoculated into Arık soil (Figure 4) on TOC content were examined, the differences in mycorrhiza, plant pattern, and interaction values after the 1st and 2nd Trials in the data obtained were found to be statistically significant. When TOC content was examined after the cover crop trial (Trial 1), the highest values obtained were 1.44% for interaction in the M3*B application, 1.35% for mycorrhiza in the M2 application, and 1.35% for the B plant pattern. When TOC content was examined after the maize+wheat trial (Trial 2), the highest values were 1.62% for interaction in the M1*C application, 1.46% for mycorrhiza in the M1 application, and 1.44% in the B plant pattern. (Figure 3.)

The data of the infertile and eroded Avadan soil (Figure 4) were examined, and the differences in all other trials and applications except for the mycorrhiza*plant interaction and mycorrhiza factor in the averages observed in the second trial were found to be statistically significant. When TOC content was examined after the wheat trial, the highest values were obtained as 0.3% with interaction in the M2*D application, 0.26% with mycorrhiza in the M2 application, and 0.26% with plant pattern in the D plant pattern. (Figure 4.)

Examining the data obtained from experiments in three types of soil, indicated an increase is observed in the soil organic carbon content from the first to the third experiments. When the mycorrhizae inoculation effects were examined, it was observed that the effect of the selected species and indigenous mycorrhizae was positive compared to the control plants. Considering the organic carbon values in all three soils, the highest results are in Havutlu soil, and the lowest values are in Avadan soil.

The factors forming the soil organic carbon content are considered to include the plant roots remaining in the soil after each experiment, different secretions belonging to different plants, and microorganisms (mycorrhizae) as causal agents.

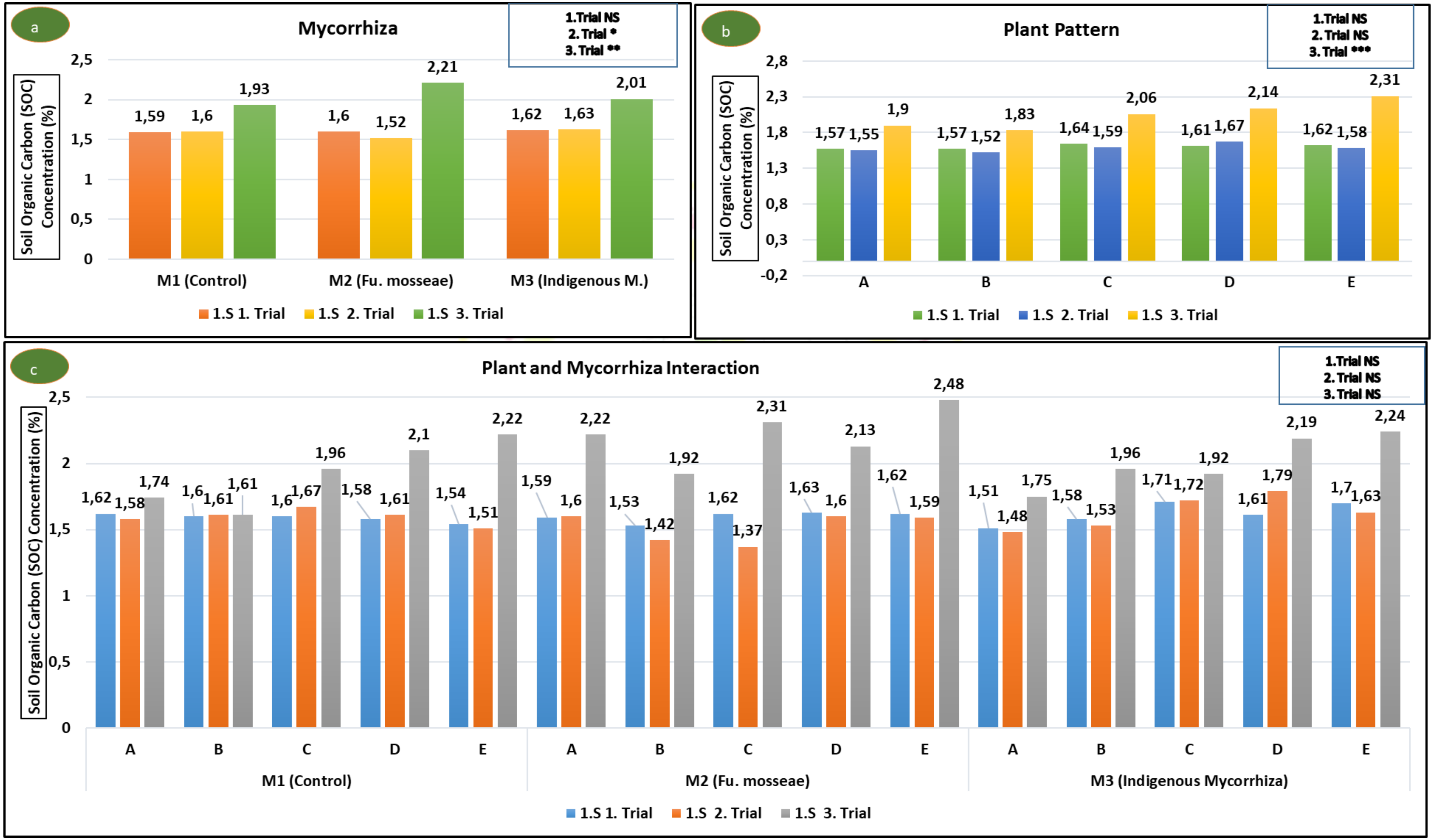

Figure 2. Change in organic carbon content in Havutlu soil, the first soil, as a result of 3 consecutive trials of different mycorrhiza inoculation and different cover crops. a: Averages of mycorrhiza applications b: Averages of plant patterns c: Averages of mycorrhiza*plant interaction NS: Non-significant *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

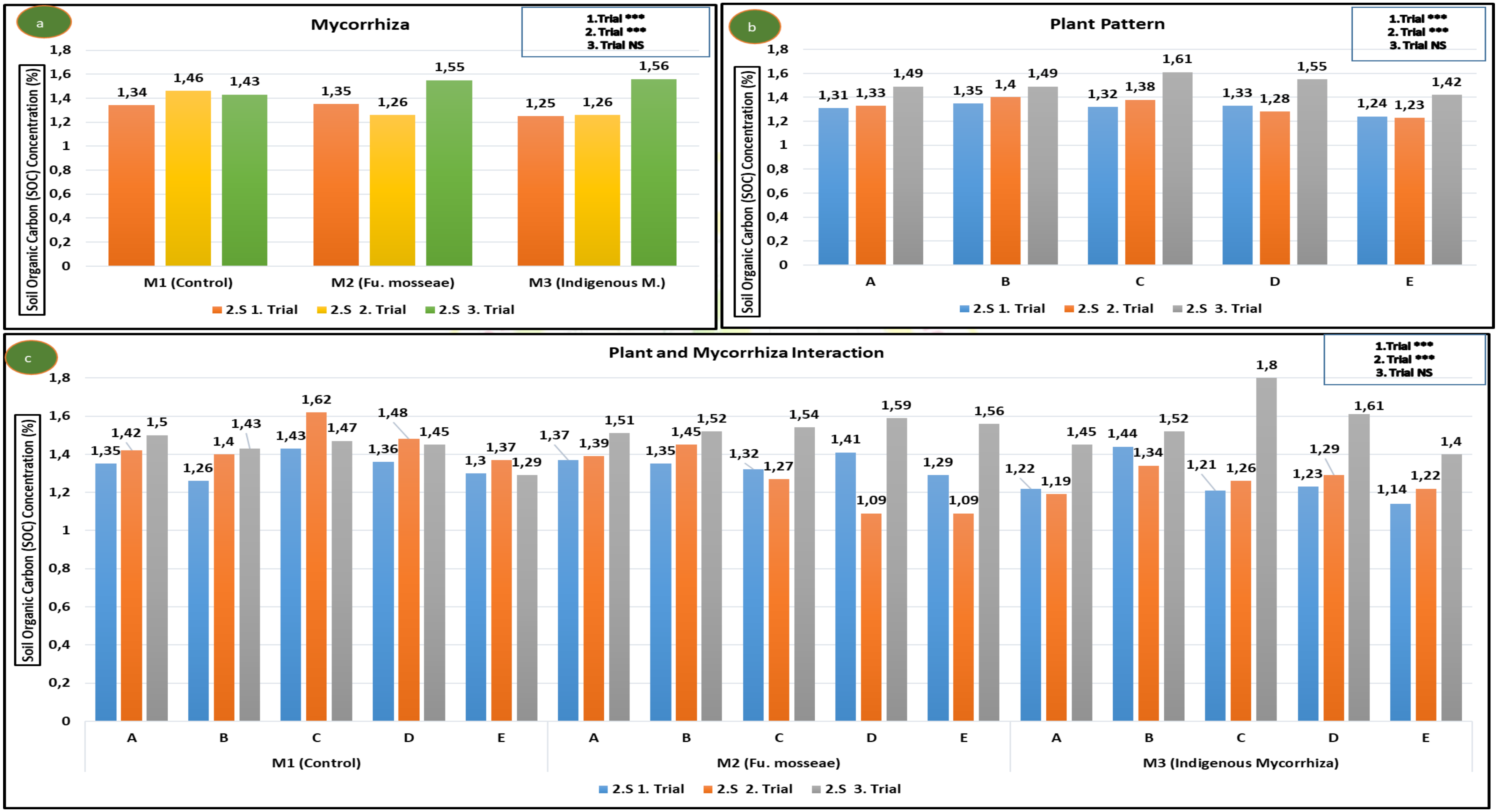

Figure 3. Change in organic carbon content in Arık soil, the second soil, as a result of 3 consecutive trials of different mycorrhiza inoculation and different cover crops.
a: Averages of mycorrhiza applications b: Averages of plant patterns c: Averages of mycorrhiza*plant interaction NS: Non-significant *: p<0.05, **:p<0.01, ***:p<0.001

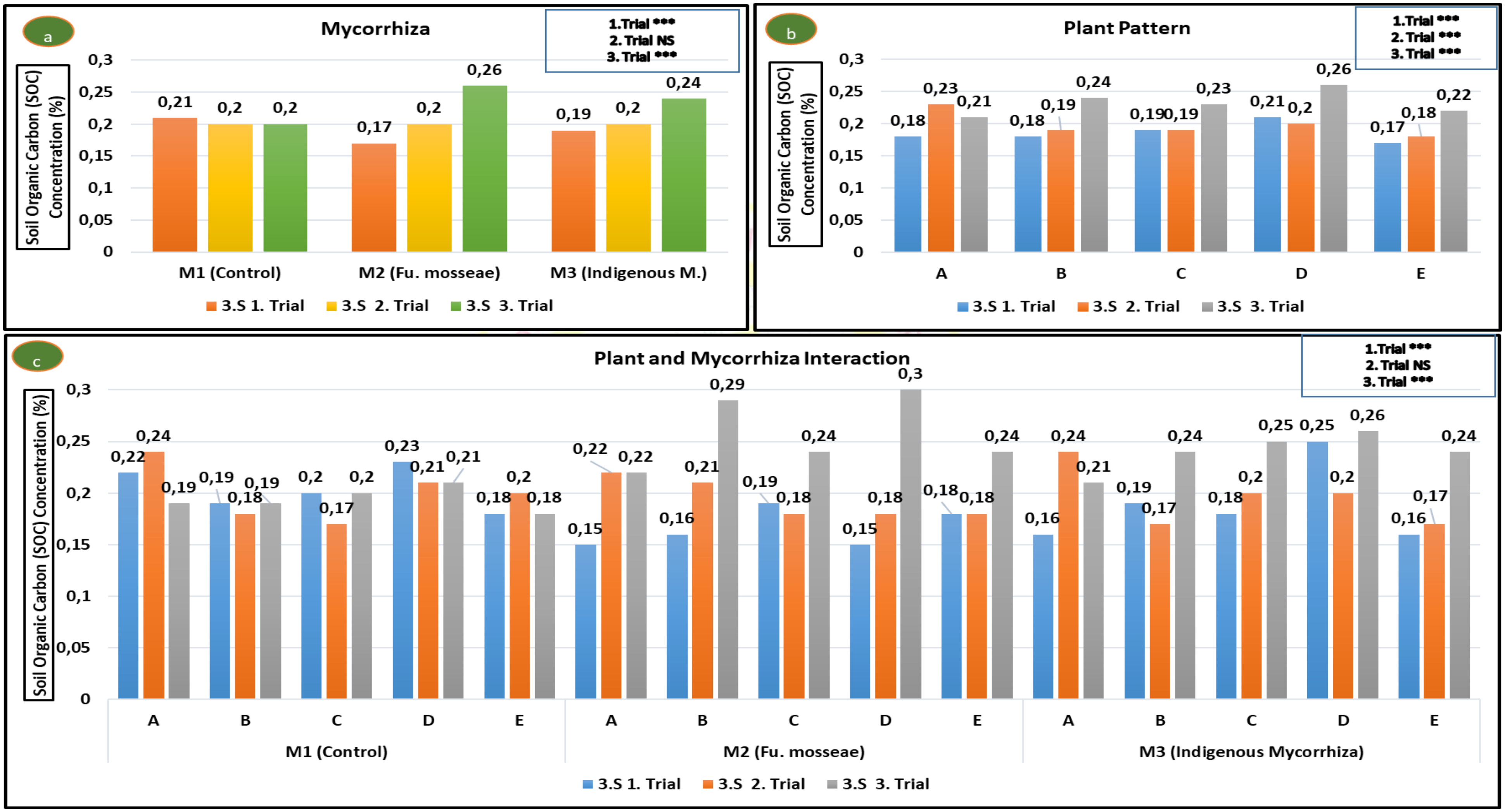

Figure 4. Change in organic carbon content in Avadan soil, the third soil, as a result of three consecutive trials of different mycorrhiza inoculation and different cover crops. a: Averages of mycorrhiza applications b: Averages of plant patterns c: Averages of mycorrhiza*plant interaction NS: Non-significant *: p<0.05, **:p<0.01, ***:p<0.001

Studies have reported that the symbiotic relation of cover crops and mycorrhizae has an important effect on increasing the SOC content. Cover crops provide organic matter to the soil through both the biomass on the surface and root residues, which then break down and form soil organic carbon. Cover crops increase microbial activities and support the carbon cycle. Cover crops strengthen the soil's organic carbon pool (Lal, 2007).

Long-term cover crop applications increase the total carbon content and active carbon forms of the soil and positively affect the nutrient cycle (Quintarelli et al., 2022; Zhang et al., 2023) Our results are supported by the data of (Leng et al., 2024), in which under long-term no-till systems, crop species diversification accumulated SOC not only on the surface but also in the whole profile. This means plant species accumulate organic carbon after root decomposition. In a different study, it was observed that cover crop plants increased the amount of easily oxidizable organic carbon in the soil. It was stated that in soils where legumes (e.g., broad beans) were used, easily oxidizable organic carbon levels were higher than those in soils with other cover crop plants. This situation was associated with the increased diversity of organic matter in the soil due to the symbiotic relationships of legumes. Furthermore, it was also reported that legume plants significantly improved soil microorganism activities (FDA enzyme activity) (Kabalan et al., 2024)

Mycorrhizae directly contribute to increasing the carbon content of the rhizosphere and mycorrhizosphere. Mycorrhizae produce glomalin-like soil proteins, which increase the physical stability of the soil and allow carbon to remain in the soil for longer periods. In addition, mycorrhizal fungi provide more carbon in the root zone of cover crops and help preserve soil organic carbon by regulating the rate of carbon decomposition. (Zhang et al., 2023).

Cover crop plants also play an important role in reducing greenhouse gas emissions through the mitigation of CO_2. Another study reported that cover crops can also reduce greenhouse gas emissions by reducing nitrate, nitrogen fixation to the soil, increasing carbon retention in the soil and reducing soil erosion. (Topçu et al., 2020). Organic carbon improves the physical condition of the soil and consequently reduces net CO_2 emissions. (Lal, 2007; Wallace et al., 2012).

To apply the research's results, it is important to test the findings with field experiments over longer periods. Moreover, our results indicate that similar experiments need to be conducted under field conditions.

CONCLUSION

After the experiments were conducted in all three soils, the data obtained were examined. It appears to be an increase in the soil organic carbon content from the first to the third experiments. When the effects of mycorrhizal inoculation were examined, the effect of the selected mycorrhizae species and indigenous mycorrhizae were positive compared to the control treatments. When the organic carbon values were obtained in all three soils, the highest results were found in the Havutlu soil, and the lowest was in the Avadan soil. Using these two applications together can be an effective strategy for increasing the organic carbon content of the soil in sustainable agricultural systems while supporting ecosystem services and combating climate change. Conducting long-term field experiments is recommended for a clearer understanding of the effect of cover crops and mycorrhizae.

CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

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