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Research Article

The Effects of Animal Manure and Mycorrhiza Applications on Soil Carbon Fractions in Tilled and Non-Tilled Conditions

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ABSTRACT

Due to long-term extensive cultivation on marginal and agriculturally suitable lands, soil organic carbon (SOC) has oxidized and released as CO_2 into the atmosphere. Furthermore, the quality of the soil and environmental safety have been further compromised by the overuse of chemical fertilizers. Despite possible trade-offs, no-till farming and the use of organic fertilizers like animal manure are advised practices to address these issues. Additionally, arbuscular mycorrhizal fungi (AMF) enhance soil aggregation and reduce the need for chemical fertilizers. However, there is limited data on the combined impact of manure and AMF on the dynamics of soil carbon in the study area, in both tillage and non-tillage scenarios. Therefore, this study aimed to investigate the effect of tillage, animal manure, and AMF application on soil carbon fractions. The study was carried out in 2023 at the Çukurova University Agricultural Research Center, Department of Soil Science and Plant Nutrition's Research Farm, Adana/Türkiye. The experiment was set up with treatments consisting of two tillage (tilled and non-tilled) as the whole plot, two AMF (with and without AMF) as the sub-plot, and two fertilizers (with and without animal manure) as the sub-sub plot factors in a split-split-plot design, totally 24 plots. Plots that did not receive animal manure received the recommended amount of mineral NPK fertilizer for optimal maize growth. Animal manure at 25 t ha^{-1} was applied in manure-treated plots. AMF inoculum was applied 50 mm under maize seeds. At harvest, soil samples were taken at 0-20 cm and 20-30 cm depths. The fractions of soil carbon (total carbon, organic, inorganic, active carbon, and particulate organic matter) were determined based on respective procedures. R computer program was used to analyze the data, and Tukey's test ($p<0.05$) was employed to compare means. The findings showed that tillage and AMF application did not significantly affect the soil carbon fractions. However, application of animal manure resulted in significant increases in total soil carbon (TC), organic carbon (OC), and particulate organic matter (POM). The study showed that the addition of fresh organic matter caused a quick rise in soil organic carbon fraction while the inorganic C remained unchanged. Given that the effects of tillage might take longer to become evident, further studies are necessary to validate these findings and better understand the long-term impacts of these soil management practices.
Keywords: Tillage, Non-Tillage, Animal Manure, Soil Carbon, Mycorrhiza

INTRODUCTION

Population growth, food and nutrition insecurity, climate change, natural resource degradation, and loss of ecosystem services are the major challenges the world is facing in the 21st century (Hall, Dawson, Macdiarmid, Matthews and Smith, 2017) . To address these challenges, a sustainably radical change in agricultural production, climate change mitigation and environmental protection, and a new green revolution are needed. Although many efforts such as intensive fertilizer use, plant breeding, and irrigation have been made to increase crop productivity, many countries are still struggling to achieve food security. It is less likely to achieve food security while putting less emphasis on climate change mitigation and environmental protection and vice versa (Mbow *et al.*, 2019; Smith and Gregory, 2012). Therefore, a 'win-win' strategy for climate change mitigation, ecosystem protection measurements, and crop yield should be ensured (Smith and Gregory, 2012). Elevated atmospheric CO_2 concentrations, have increased from about 280 ppm before the Industrial Revolution to 420 ppm today (Ortas, 2022), contribute to temperature increases that severely impact human society. Soil, as the largest carbon reservoir in terrestrial ecosystems, holds significantly more organic carbon than the atmosphere and vegetation. However, soil and organic matter degradation leads to CO_2 release, affecting atmospheric carbon levels(Ortas, 2022). Small changes in soil carbon storage can significantly alter atmospheric CO_2 concentrations (Rustad, Huntington and Boone, 2000; Sanderman, Hengl and Fiske, 2017; Stockmann *et al.*, 2013). Enhancing soil carbon sequestration is therefore a crucial strategy for mitigating global climate change and addressing food security (Lal, 2004). Soil carbon (C) is a key factor influencing the sustainability of agricultural systems, with alterations possible in both total and labile carbon pools (Blair, Lefroy and Lisle, 1995).

Research is being conducted on modifications to agricultural practices aimed at mitigating climate change and addressing pervasive soil degradation to enhance food security, promote environmental conservation, and achieve sustainability (Srinivasarao, Lal, Kundu and Thakur, 2015). Given that soil organic carbon (SOC) concentration significantly influences soil physicochemical properties and biological activity, the sequestration of carbon in agricultural soils necessitates the adoption of revised management practices (Srinivasarao, Lal, Kundu and Thakur, 2015). Soil organic matter (SOM) is a heterogeneous and dynamic entity, varying in carbon content, molecular structure, decomposition rates, and turnover times (Oades, 1988). Current SOM studies typically classify it into pools based on intrinsic decomposition rates and influencing factors, such as particulate organic carbon and active or $KMnO_4$ oxidizable carbon (Blair, Lefroy and Lisle, 1995). These carbon fractions are more responsive to management practices than total soil organic carbon and may indicate future changes in total SOC stock (Tong *et al.*, 2014), though they often show weak associations with measurable quantities (Six *et al.*, 2002).

Conventional soil and crop management practices, including intensive tillage, extensive mineral fertilizer use, and long-term monoculture, significantly reduce SOC levels (Kumar, Kadono, Lal and Dick, 2012; Lal, 2019; Ortas, 2019). Soil aggregate formation is crucial for SOC accumulation (Bronick and Lal, 2005; Ortas and Lal, 2012; Six, Elliott and Paustian, 2000; Zhang *et al.*, 2023). However, heavy tillage, excessive fertilizer use, and over-irrigation degrade carbon-binding aggregates. Organic matter within macro-aggregates decomposes more slowly due to reduced microbial accessibility (Lützow *et al.*, 2006). Tillage disrupts these macro-aggregates, accelerating the decomposition of protected organic matter (Six, Elliott and Paustian, 1999).

Mycorrhizal infection enhances soil physical properties, thereby improving plant health. This positive impact on plant growth and biomass is anticipated to increase carbon sequestration (Smith and Read, 2010). Excessive soil tillage, burning of crop residues and improper fertilizer application can diminish mycorrhizal symbiosis, negatively impacting soil and plant quality due to adverse effects on soil biological organisms (Ortas and Coskan, 2016; Ortaş, Lal and Kapur, 2017). This intern can affect the soil C storage potential. Moreover, the use of organic fertilizers like manure and compost can boost soil C and biological activities compared to conventional mineral fertilization. Despite these facts, there is limited data on the combined impact of manure and AMF on the dynamics of soil carbon in the study area, in both tillage and non-tillage scenarios. Therefore, this study aimed to investigate the effect of soil management practices namely tillage, animal manure, and AMF application on soil carbon fractions.

MATERIALS AND METHODS

Site Descriptions and Experimental Setups

The study was carried out in 2023 at the Çukurova University Agricultural Research Center, Department of Soil Science and Plant Nutrition's research farm, Adana/Turkiye. The experiment was set up with treatments consisting of two tillage (tilled and non-tilled) as the whole plot, two AMF (with and without AMF) as the sub-plot, and two fertilizers (with and without animal manure) as the sub-sub plot factors in a split-split-plot design, totally 24 plots (each 21.25 m²). Plots that did not receive animal manure received the recommended dose of mineral NPK fertilizer for maize. Animal manure at 25 t ha^{-1} was applied in manure-treated plots. AMF inoculum (500 spores per row) was applied under maize seeds.

Soil Sampling and Analyses

At harvest, soil samples were taken at 0 to 20 cm and 20 to 30 cm depths. The fractions of soil carbon (total carbon, organic, inorganic, active carbon, and particulate organic matter) were determined based on respective procedures. The total soil C was determined by the dry combustion method, and SOC is the difference between total carbon and inorganic carbon. Inorganic carbon (IC) in the soil was measured using a calcimeter as described by (López-Bucio *et al.*, 2002). About 0.5 g of dry soil was treated with 10 ml of HCl, and the resulting gas was analyzed using a Scheibler gas-resistant apparatus. Particulate organic matter (POM) was determined by washing a mixture of soil and organic matter to remove clay and silt fractions through wet sieving with a 53 µm sieve. The sand and organic matter remaining on the sieve were carefully collected and dried at 55°C for 36 hours and weighed. The dry sample was weighed and put into the furnace for 4 and half hour at 450°C, and the POM was estimated using the weight loss on ignition procedure as described by (Nciizah and Wakindiki, 2012).

Statistical Analysis and Data Evaluation

R computer program was used to analyze the data, and Tukey's test ($p<0.05$) was employed to compare means. Correlation analysis was also done to investigate the relationship between soil C fractions.

RESULTS AND DISCUSSIONS

Soil carbon fraction and their relationships

Soil carbon (C) can be primarily classified as organic and inorganic C. In terms of their stability, formation and function, the soil organic carbon can be further fractionated into dissolved, mineral-associated, particulate, active or permanganate oxidizable, and microbial biomass carbon (Hu *et al.*, 2023). In this study, the total carbon, inorganic carbon (SIC), organic carbon (SOC), particulate organic matter (POM), and active carbon (AC) fractions were studied. Moreover, the Pearson correlation analysis was performed to determine the association among these C fractions. The correlation result reveals that most of the soil carbon fractions were positively associated. Soil organic carbon showed a significantly strong association (p<0.001) with POM and total soil C at 0-20 cm depth (Figure 1). However, SIC and organic carbon fractions including SOC, POM and AC had a weak negative correlation as observed in other studies (Somenahally *et al.*, 2023; Wang, Wang and Feng, 2023; Zhao, Zhang, Cao and Tan, 2019).

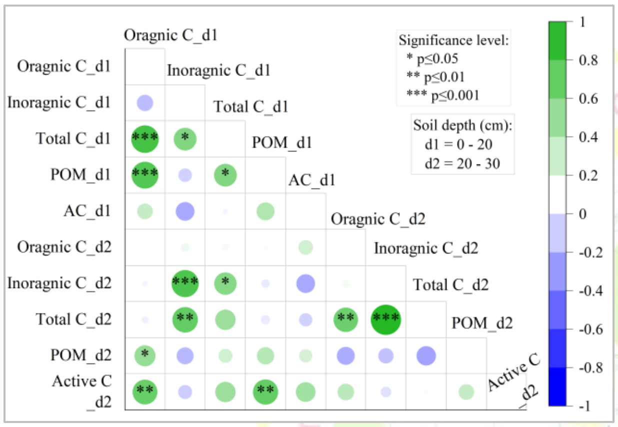

Figure 1. Pearson correlation between soil carbon fractions at 0-20 and 20-30 cm depths. Where, C: is carbon, POM: is particulate organic matter, and AC: active soil carbon.

Total Soil Carbon

The total carbon (TC) in the soil includes all fractions of carbon including inorganic fractions like carbonates, and organic fractions. The results presented in Figure 2 show the effects of tillage, animal manure and AMF treatments on soil total carbon. At 0-20 cm depth, the effects of animal manure application and its interaction with tillage were significant, while other factors were not significant (Figure 2). Total carbon in the soil was significantly higher with manure application (5.60%) compared to the non-manure treatment (5.44%). This difference may be attributed to the incorporation of organic materials from manure, which tends to improve soil structure and nutrient availability, thereby promoting carbon retention. However, no significant variation was noted on soil TC at the 20-30 cm depth, suggesting that manure application has more pronounced effects in the upper soil layer and does not extend to deeper layers during the first-year application. In support of this finding, Bridges, Das, Neikirk and Lal (2023) reported that despite slight statistical differences between fields that used manure and those that did not, increasing tillage intensities did not significantly affect soil TC. Furthermore, since the soil TC is primarily composed of inorganic fractions (Somenahally *et al.*, 2023), practices that affect OC might not have a major impact on it, at least in the early stages of the trial.

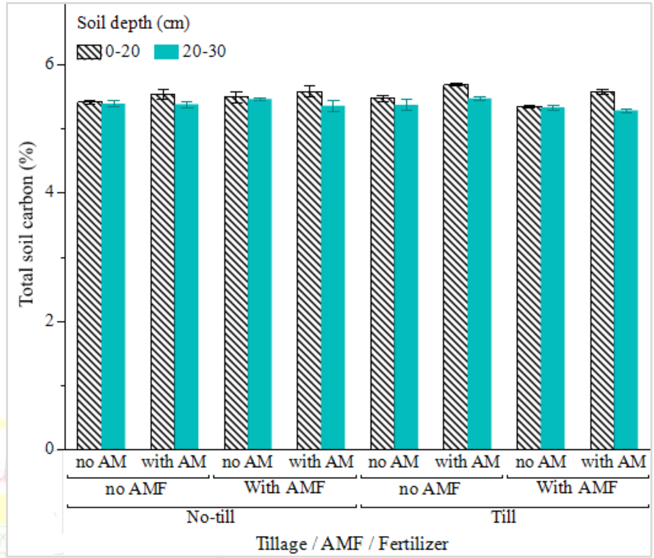

Figure 2. Effect of tillage, AMF and manure application on total carbon soil. AMF: Arbuscular mycorrhizal fungi. The error bar represents ± standard error of the means (n= 3).

Soil Inorganic Carbon

The soil inorganic carbon (SIC) is the carbon associated with carbonates, primarily calcium carbonate. In this study, SIC constitutes at least 80% of the total soil C. The correlation analysis indicated a highly significant (p<0.001) positive relationship between TC and SIC (Figure 1). However, this study revealed that tillage, manure, and AMF treatments did not have significant effects on SIC, except for a slight variation in soil depth, as shown in Figure 3. Relatively higher SIC was observed at 20-30 cm depth compared to the surface soil. A similar result was reported by Öztürk and Ortaş (2024) from a long-term tillage experiment in the same soil series. This could be due to the leaching of carbonates from the upper soil layer and their accumulation at bottom depths. Additionally, the release of root exudates and the associated weak organic acids can facilitate the dissolution of carbonates near the surface.

Soil Organic Carbon

This study shows that the soil organic carbon (SOC) fractions constitute less than 20% of the TC regardless of the treatments and soil depth. The results show that AMF did not change the SOC level. Although numerous other studies have reported a significant effect of tillage on SOC (Bono, Alvarez, Buschiazzo and Cantet, 2008; Haddaway *et al.*, 2016), this study found no significant differences between tilled and no-tilled soil. However, the application of animal manure resulted in a rapid and significant increase in SOC at a depth of 0-20 cm. At this depth, the SOC was significantly higher (1.19%) under animal manure application relative to non-manure treatment (0.99%), a 20% increase in SOC (Figure 4).

Similarly, in a long-term trail manure application increased the SOC by 44% compared to mineral fertilization (Akşahin, Işik, Öztürk and Ortaş, 2021). An average increase of SOC by 35.4% under manure application was reported (Gross and Glaser, 2021). Manure can enhance SOC, primarily by increasing the labile carbon fractions (Zhang, Zhao, Li and Zhang, 2022). Many other studies also reported a positive impact of manure on SOC. Long-term studies indicate a linear correlation between manure application and SOC content, where each ton of manure organic carbon added results in an increase of SOC by approximately 0.02 % in the surface soil (Hao, Chang, Travis and Zhang, 2003).

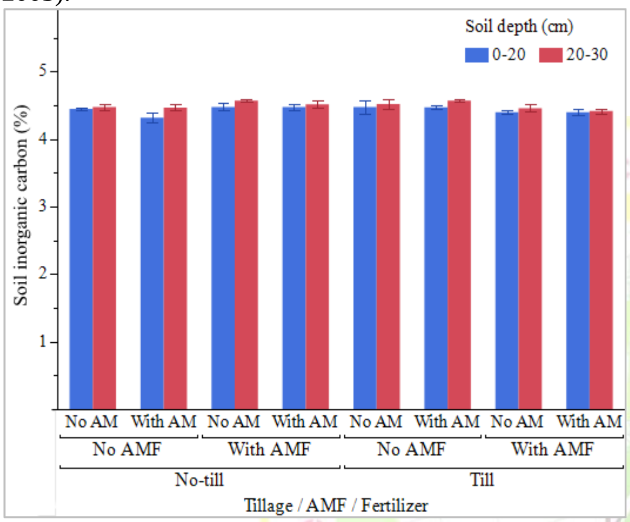

Figure 3. Effect of tillage, AMF and manure application on inorganic Carbon. AMF: Arbuscular mycorrhizal fungi; AM: animal manure. The error bar represents ± standard error of the means (n= 3).

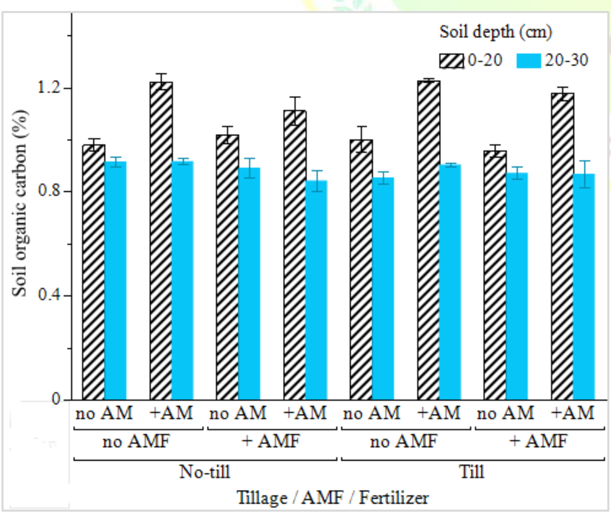

Figure 4. Effect of tillage, AMF and manure application on soil organic carbon. AMF: Arbuscular mycorrhizal fungi. *+: added; AM*: animal manure. The error bar represents ± standard error of the means.

The impact of tillage, manure and AMF treatments on SOC at 20 -30 cm depth was statistically non-significant. There was a slightly higher SOC at the surface depth compared to the 20 to 30 cm depth. This might be attributed to higher organic C input at surface soil than lower soil depth. Moreover, tillage may improve the mixing and distribution of organic material at topsoil. However, prolonged, and intensive soil tillage can further degrade this SOC through increased oxidation as indicated in a long-term tillage experiment (Öztürk and Ortaş, 2024).

Particulate Soil Organic Matter

Particulate organic matter (POM) can be defined as the component of soil organic matter with a particle size exceeding 53 µm. POM exerts a more significant impact on soil properties, especially aggregate stability, than other fractions. The incorporation of substantial organic fractions, including partially decomposed organic matter such as manure, compost and crop residues, enhances the POM. The results of this study demonstrate that manure significantly affected POM, while tillage and AMF did not affect, and similar trends were observed at 0-20 and 20-30 cm soil depths. The highest POM of 3.78 and 2.67 g POM kg^{-1} soil were observed under manure application with tillage and AMF inoculation at 0-20 and 20-30 cm depth, respectively (Figure 5). POM was higher at the top surface than the 20-30 cm depth. Manure applications increase POM by 51% at 0-20 cm and 52% at 20-30 cm compared to conventional chemical fertilizer applications. This result is consistent with other findings (Gautam, Guzman, Kovacs and Kumar, 2022; Kauer, Pärnpuu, Talgre, Eremeev and Luik, 2021; Mando *et al.*, 2005) which reported that application of manure enhances the content of POM and total SOC, particularly at higher application rates.

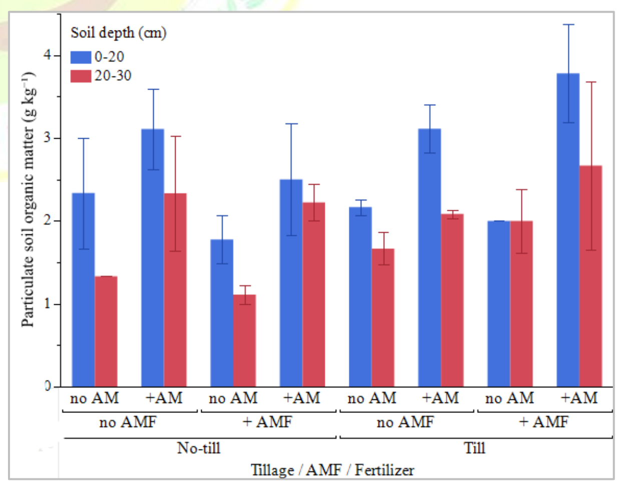

Figure 5. Effect of tillage, AMF and manure application on particulate soil organic matter. AMF: Arbuscular mycorrhizal fungi; *+: added; AM*: animal manure. The error bar represents ± standard error of the means (n= 3).

Active Soil Carbon

Active soil carbon or permanganate oxidizable carbon (POC) is the fraction of SOC that is readily available for biological processes in the soil. Compared to POM, this C faction is more biologically dynamic and less stable. POC fails to differentiate between labile and non-labile carbon under specific conditions (Tirol-Padre and Ladha, 2004). Despite this limitation, POC is a sensitive indicator of the effects of tillage and organic inputs on soil carbon (Gruver, 2015). Figure 6 illustrates the findings of this study.

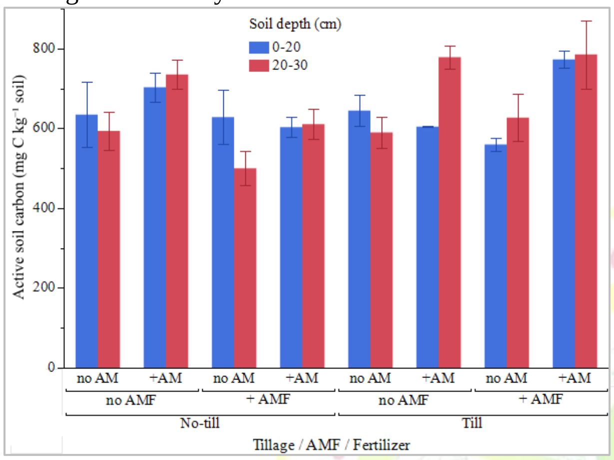

Figure 6. Effect of tillage, AMF and manure application on active soil carbon. AMF: Arbuscular mycorrhizal fungi; *+: added; AM*: animal manure. The error bar represents ± standard error (n= 3).

Similar to other SOC fractions, POC was also highly influenced by animal manure application rather than tillage and AMF (Figure 6). At 0-20 cm depth, significantly the highest POC (772.69 mg kg^{-1}) was obtained with manure application under tilled and AMF conditions, while the lowest value (559.69 mg kg^{-1}) was observed in none-manure treated plots at the same soil depth and similar conditions (Figure 6). Unlike other soil carbon fractions, active soil carbon is relatively higher at the 20–30 cm depth compared to the surface soil. The dynamic nature and rapid utilization of this faction by soil microbiotas in the surface layer may be the primary reason for its lower value.

CONCLUSIONS

Soil management practices such as tillage and fertilization play a key role in influencing the soil carbon pool and dynamics. This study aimed to investigate the effects of different treatments specifically, tillage, the use of mycorrhizal fungi, and animal manure application on various soil carbon fractions, including total, organic, inorganic, active and particulate carbon fractions, at 0-20 and 20-30 cm soil depths. The result revealed that the soil inorganic carbon, which accounts for more than 80% of TC, remains unchanged across the different treatments. However, soil organic C fractions such as SOC, POM and active carbon showed significant improvement with the application of animal manure, while tillage and AMF did not have notable impacts on the increase of SOC contents. Moreover, it is important to consider the effect of AMF on soil aggregation and the SOC fractions in various soil aggregates. The improvement in SOC, POM, and active carbon following the addition of manure suggests that organic amendments can quickly boost these carbon fractions in the soil. On the other hand, the potential effects of tillage on the turnover rate of soil C may require a longer time to become evident. However, since this research was conducted over just one cropping season, further studies are necessary to validate these findings and better understand the long-term impacts of these soil management practices.

CONFLICT OF INTEREST

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

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