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EFFECTS OF LEAVES AND LITTER OF *OLEA EUROPAEA* L. ON SOIL ORGANIC CARBON MINERALIZATION: A LABORATORY STUDY

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1. INTRODUCTION

Soil microorganisms are unique organisms that are important players in the processes of decomposition, mineralization and immobilization of all plant and animal residues (Figure 1). These microorganisms regulate the processes of organic carbon inputs and outputs in the soil ecosystem. It's essential to interpret the behaviours of the soil microorganisms to gain better understanding in the soil carbon sequestration (Brookes et al. 2008).

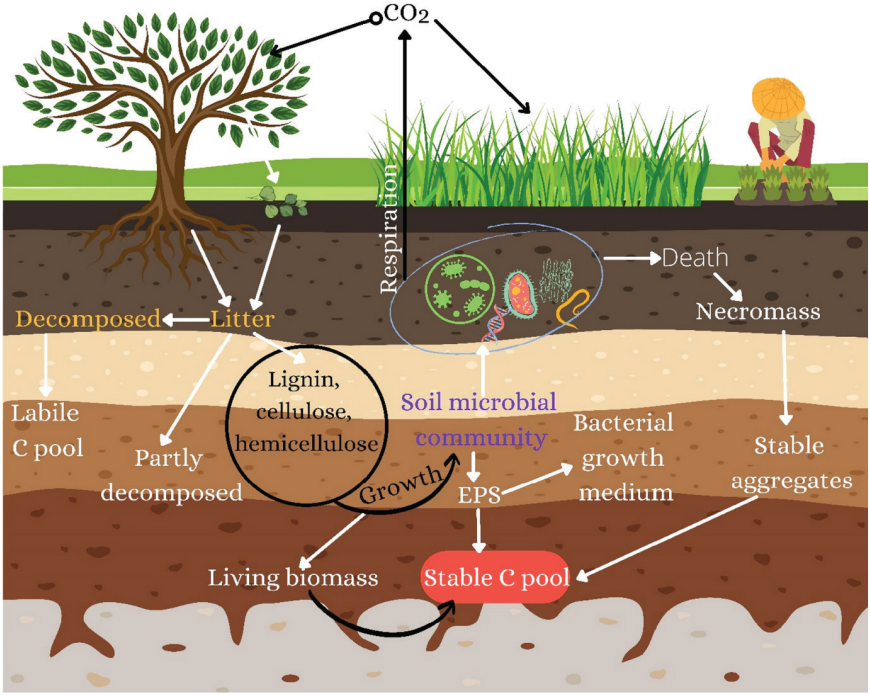


Figure 1 *Relations of soil microbial communities with distinct carbon pools in the soil ecosystems (Bhattacharyya et al., 2022).*

Soil microorganisms sustain their lives by utilizing the two fundamental carbon sources to obtain energy: 1) inputs of fresh plant and animal residues 2) humified soil organic matter (Figure 1). The most readily available and energy producer materials per unit carbon are the fresh biological organic inputs. Large molecules like cellulose and protein are efficiently break down and converted into small subunits like monosaccharide and amino acid that will be assimilated by the microbial cells (Figure 1). During this process, between 40% and 60% of organic carbon in these substrates transformed into CO_2. Soil organic matter fundamentally occur as a result of the functions of soil microorganisms on these fresh inputs. With the continuous recycling of microbial biomass, microorganisms are eventually converted into biological carbon forms

whose half-lives are more than centuries (Jenkinson et al. 1992).

Organic matter stored in the soil is structurally different from organic materials that are freshly introduced to the soil while it has complex chemical structure. This structure can be changed in size and consists of large number of non-sequential molecules and many various functional groups such as carboxyls, fenolic and alcholic hydroxyls, carboniles, amino (Tate III, 1992). Due to irregular reactions catalyzed by the active enzymes in the dead cells and random condensations of reactive products generated from the decomposition of compounds including quinone, lignin, cellulose and nitrogen, these molecules have no stable polymeric characterization which biological macromolecules have (Stevenson, 1994). Deficiency of polymeric structure has an active role in the resistance of soil organic matter to microbial decomposition and the physical protection of organic matter (Watts et al., 2001). However, soil microbial biomass, which survive after months and even years, can slowly mineralize soil organic matter and CO_2 occurs slowly in this process (Figure 2) (Joergensen et al., 1990). High amounts of organic carbon are globally sequestrated in the soils and estimation of this amount was reported as 1400 gigatons at 1 m soil depth (Post et al., 1982). Thus, this magnificent transformation process contributes largely in the Earth's carbon cycle and release of greenhouse gas emissions such as CO_2, NO etc. (Figure 2).

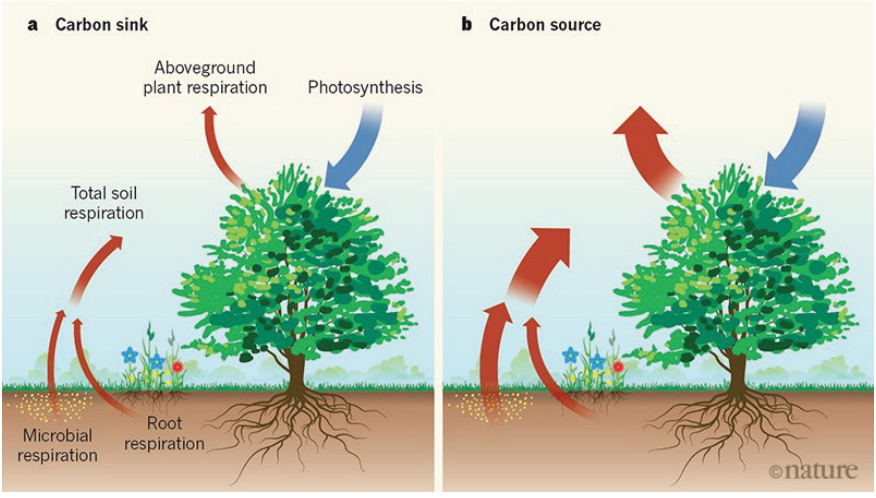


Figure 2 *Carbon fluxes at Earth's surface a. as carbon sink b. as carbon source (Ogle, 2018).*

Two of the main pathways of carbon dioxide release are soil and plant respirations in the terrestrial ecosystems (Glanville et al. 2012). It's noted that soils contain 1500 Pg C pool size which is the twice of atmospheric pool size and global flux from soil respiration was estimated as 73 Pg C

y^{-1} which is more than other terrestrial-atmospheric carbon releases except for gross primary production (Schlesinger and Andrews, 2000; Rustad et al., 2000). Roots of plants and microbial decomposition of organic matter in the soil produce CO_2. Carbon release from soil relies on many soil factors (soil moisture, temperature, pH etc.), plant factors (vegetation communities, root density, litter inputs etc.) and alterations by human intervention (heavy metal accumulation, land use management, pollution etc.) (Raich and Tufekcioglu, 2000). The availability of labile carbon substrates in the soil generally restraint the soil microbial activities and respiration (Demoling et al., 2007), which these substrates are originated from root exudates (Jones et al., 2009) and turnover of dead roots (Gill and Jackson, 2000).

It's reported that area of olive gardens is 10.5 ha in the worldwide (Figure 3) (FAO, 2021). Olive trees have low fruit yields due to water deficiency and in particular they are well adapted to arid regions like areas under Mediterranean climate. Olive is a natural evergreen and essential fruit tree in the Mediterranean Region (Malamidou et al., 2018). Olive trees contribute an important amount of litter to the soil ecosystems and 90% of this amount consists of its leaves (Stevenson, 1994).



Figure 3 *Worldwide distribution of olive trees (Cimato and Attilio, 2011)*

Soil organic carbon (SOC) turnover can be increased in short term by adding an external organic substrate and this is called as "priming effect" (Kuzyakov, 2010). Priming effects of plant materials or easy decomposable substances on soil have been widespread studied which are represent for the organic carbon inputs in the natural ecosystems (Wang et al. 2014). However, it has been reported that directions of priming effects are inconsistent with each other in the different experiments and when organic carbon introduced into the soil, positive priming (Fontaine et al., 2007; Zhang & Wang, 2012), negative priming and neutral priming effects were

found (Hamer & Marschner, 2005; Nottingham et al., 2009). Plant litter is the main source of soil organic carbon in the forest ecosystems and it can change the rate of SOC mineralization (Wang et al., 2014).

In this study, effects of olive leaves and litter on SOC mineralization in the olive trees (*Olea europaea* L., Oleaceae) that naturally grow in Cukurova University Campus (Adana, Southern Turkey). Olive leaves and litter were added to the soil at 0.5% and 1% and these soil+plant material mixtures were humidified at 80% of field capacity and incubated at 28°C for 52 days.

2. MATERIAL AND METHODS

2.1. Material and Study Area

Soil, leaf and litter samples of olive (*Olea europaea* L.) have been taken from a natural olive grove in Cukurova University Campus, Adana (37°3'26" N, 35°21'19" E), Turkey, in October 2021. Mean annual precipitation and temperature in Adana were 659 mm and 19.1°C for 35 years and this region had the semi-arid Mediterranean climate (Kum and Celik, 2014).

After air drying, olive soils were sieved through a 2 mm mesh sieve and olive leaves and litter were powdered before analysis. Soil texture, soil pH and soil field capacity were determined by Bouyoucos hydrometer, a 1:2.5 soil-water suspension with pH-meter (inoLab pH/Cond 720, WTW GmbH, Weilheim, Germany) and 1/3 atmospheric pressure with a vacuum pump, respectively (Kacar, 2012). Organic carbon and total nitrogen concentrations in the olive soil, leaf and litter samples were measured by using a modified Walkley and Black method and Kjeldahl method, respectively (Kacar, 2012).

2.2. Soil Carbon Mineralization and Kinetics

Olive leaves (0.5% Leaf and 1% Leaf) and litter (0.5% Litter and 1% Litter) were mixed with its soil at 0.5% and at %1 doses. For this purpose, 100 g soil and plant material were first mixed and then placed in 750 ml incubation vessels. These mixtures were humidified at the 80% of soil field capacity and then were incubated at 28°C for 52 days. Soils untreated with leaves or litter were used as control. As a result of soil microbial activity, CO_2 was trapped periodically by 10 ml 1 M NaOH solution in beakers which are placed in incubation vessels. Amount of respired CO_2 was determined with the titration with 1 M HCl in the incubation vessels. Three replicates were used for each treatment and control. After 52 days of incubation, all measured CO_2 during the incubation were summed up to calculate cumulative carbon mineralization (C_{min}, mg CO_2-C/100 g soil) and these values were divided by soil organic carbon level to

obtain the rate of C_{min} (Koçak and Darıcı, 2016). A first order kinetic model proposed by Ajwa and Tabatabai (1994) was used to simulate the relationship between the cumulative C_{min} quantity and incubation time in all treatments: $C_{min} = C_0 (1 - e^{-kt})$. C_{min} is the amount of cumulative carbon mineralization through incubation period (days) while C0 is the quantity of potentially mineralizable carbon in the soil and k (day^{-1}) is the mineralization rate constant.

2.3. Statistical Analysis

The data obtained from the laboratory incubation were submitted to ANOVA to show the differences between treatments by using the SPSS v.20 software. The separation of averages of control and treatments were made by using Tukey's HSD and differences between the means of control and treatments were declared as significant at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Some Properties of Olive Soil, Leaf and Litter Samples in Cukurova University Campus

Some physical and chemical properties of olive soils and organic carbon and total nitrogen contents of leaves and litter were given in Table 1. Olive soils were sandy clay loam and slightly basic. Organic carbon, total nitrogen contents and C/N rate of soils were 4.23%, 0.520% and 8.13, respectively (Table 1). Koçak and Darıcı (2022) reported that natural olive soils in Cukurova University Campus were clay at 0-10 cm depth while organic carbon contents of soils were between 2.68% and 3.87%, total nitrogen contents of soils were between 0.179% and 0.378%, C/N rates were between 10.46 and 16.28 between 2013-2015. Sağlıker and Darıcı (2005) claimed that natural olive soils were sandy loam at 0-10 cm soil depth in Cukurova University Campus while organic carbon contents of these soils were between 1.99% and 3.31%, total nitrogen contents of soils were between 0.19% and 0.33% and C/N rates were between 11.4 and 11.7 between 1999-2000. Differences in results between the current study and past studies may be caused by different sampling times and sampling trees.

Organic carbon and total nitrogen contents of olive leaves and C/N of leaves were 42.03%, 1.340% and 31.35, respectively (Table 1). These contents and C/N of litter were 31.09%, 0.753% and 41.31, respectively (Table 1). Sağlıker and Darıcı (2005) indicated that organic carbon contents and total nitrogen contents of natural olives leaves were between 35.4% and 47.6% and between 1.15% and 1.77% in Cukurova University Campus, respectively. In the latter study, organic carbon and total nitrogen contents of litter were between 30.0% and 46.9% and between 0.87% and 1.36% (Sağlıker and Darıcı, 2005). Malamidou et al. (2018) noted that

organic carbon and total nitrogen contents (mg g^{-1}) of leaves in a olive grove were 520 and 15.0, respectively. Obtained results of litter and leaves were consistent with these studies.

Table 1 *Some properties of olive soil, leaf and litter samples in Cukurova University Campus (mean±standard error, n=3)*

Olea europaea L. (Olive)				
	Clay (%)	28.67	±	0.68
	Silt (%)	22.82	±	1.79
	Sand (%)	48.51	±	1.18
	Texture	Sandy Clay Loam		
Soil	Field Capacity (%)	37.60	±	0.26
	pH	7.27	±	0.08
	Organic Carbon (%)	4.23	±	0.05
	Total Nitrogen (%)	0.520	±	0.020
	C/N	8.13	±	0.08
	Organic Carbon (%)	42.03	±	1.36
Leaves	Total Nitrogen (%)	1.340	±	0.017
	C/N	31.35	±	0.64
	Organic Carbon (%)	31.09	±	0.75
Litter	Total Nitrogen (%)	0.753	±	0.015
	C/N	41.31	±	1.48

3.2. Soil CO_2 Evolution and Kinetics

The biogeochemical C cycle have an active role in the linkages between plant vegetation and soil ecosystems (Regina and Tarazona, 2000). One of the main significant processes in the regulation of functioning of natural and agricultural ecosystems is the soil organic carbon mineralization (Johnson, 1995). The nutrient flux in the soil is controlled by the rate of carbon mineralization because the energy sources constrain nutrient availability in the soil to maintain the populations of soil microorganisms (Núñez et al. 2001, Saggar et al. 2001). Prediction of carbon mineralization rate is an easy method to control how C stored in terrestrial ecosystems reacts to changes occurred in vegetation or climate because most of the detrital carbon stored in terrestrial ecosystems can be found in mineral soil (Giardian et al., 2001). Thus, evaluation of biological process of ecosystem evolution requires the scientific modelling of soil C mineralization and its pathway in a dynamic environment (Su et al. 2004).

Cumulative carbon mineralizations [mg $C(CO_2)$/100 g soil] in the soils added with olive leaves (0.5% and 1%) and litter (0.5% and 1%) were between 167.46 (control) and 326.07 (1% leaf) in the end of incubation period (Figure 4). Addition of olive leaves and litter in all doses significantly increased soil microbial respiration compared to control ($P<0.05$). However, there

were found no significant differences between the same doses of leaves and litter in the cumulative carbon mineralization.

Rates of carbon mineralizations were between 3.96% (Control) and 7.02% (1% leaf and 1% litter) after 52 days of incubation (Figure 5). C mineralization rates were significantly increased by the addition of olive leaves and litter ($P<0.05$, Figure 5). However, no significant differences were found between the same doses of leaves and litter in rate of carbon mineralization.

Kocak and Darıcı (2016) added the leaves of bay leaves (*Laurus nobilis* L.) to its soil at the half and same amount of soil organic carbon and measured the carbon mineralization under laboratory conditions. After 54 days of incubation, addition of leaves to soil significantly enhanced soil organic carbon mineralization ($P<0.05$). Cenkseven et al. (2017) added the leaves of oleander (*Nerium oleander* L.) to its soil at the half, same and twice of soil organic carbon concentration and measured the carbon mineralization. Addition of oleander leaves stimulated soil organic carbon mineralization in two levels of field capacity (60% and 80% of field capacity)

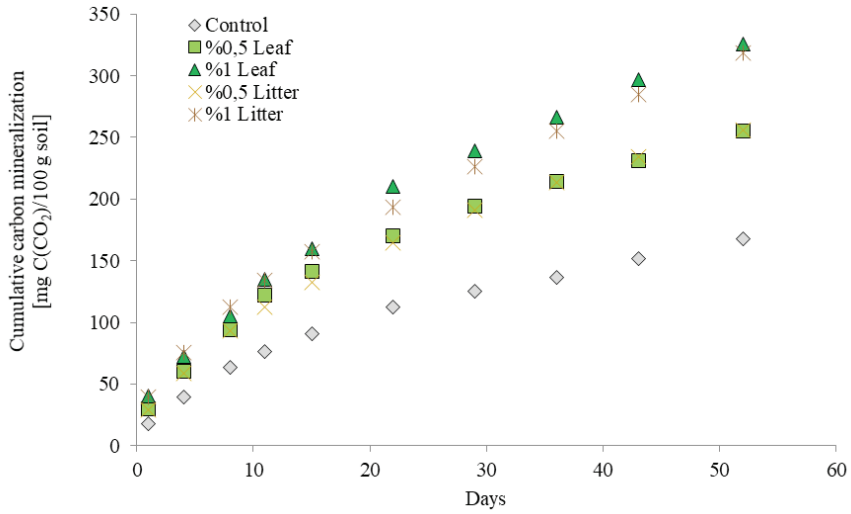


Figure 4 *Cumulative carbon mineralizations in the soils added with olive leaves and litter at 0.5% and 1% (n=3)*

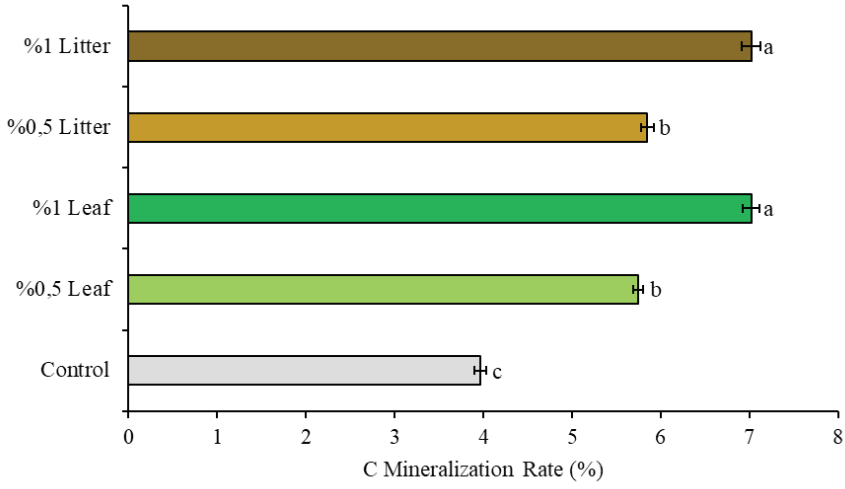


Figure 5 *Rates of carbon mineralization in the soils added with olive leaves and litter at 0.5% and 1%. Each column in graphs represents the mean of three replicates ± standard. Lowercase letters denote significant differences between treatments based on Tukey's HSD (P<0.05).*

In general, carbon mineralization dynamics in incubation studies can be expressed as a mathematical equation to predict the ability of soils in providing potential mineralizable organic carbon and to determine the balance of organic matter (Riffaldi et al., 1996). One of the descriptive ways of soil organic carbon mineralization is the usage of the first-order equation. This model was originally suggested by Jones (1984) for nitrogen mineralization and includes a parameter that defines a pool of decomposable substrate in a easy way which produce a mineralization flush (C_0) in the first incubation interval. In addition, this model is one of the most suitable kinetic models that describe the decomposition and mineralization of various organic material types (Saviozzi et al. 1993). In the current study, potentially mineralizable carbon (C_0, mg $C(CO_2)$ 100 g^{-1} soil) values obtained by the first-order equation ranged from a minimum of 169.17 for control to a maximum of 355.91 (1% leaf) in Table 2. In addition, carbon mineralization constant rates ($days^{-1}$) differed among the treatments and all obtained values in this study were ranged between 0.041 (1% leaf) and 0.054 (0.5% leaf) in Table 2. These k values in the current study indicated that organic materials metabolized in the microbial respiration were similar or had the same levels of availability (Riffaldi et al., 1996).

Table 2 *Parameter estimates according to the first-order equation model for carbon mineralization in the olive soils added with olive leaves and litter (C_0: potentially mineralizable carbon in the soil, C_{min}: mineralized soil carbon at the end of the incubation period, k: mineralization rate constant)*

	C_0	C_{min}	k
	mg $C(CO_2)$/100 g soil		$days^{-1}$
Control	169.17	167.46	0.052
0.5% Leaf	257.91	254.91	0.054
1% Leaf	355.91	326.07	0.041
0.5% Litter	269.68	256.23	0.047
%1 Litter	337.66	318.29	0.043

4. CONCLUSIONS

Soil microorganisms are key players in the mineralization of soil organic matter. Heterotrophic microorganisms require the organic materials such as litter, roots and their exudates to sustain their lives in the soil ecosystems. Decomposition of plant materials in the soil relies on the abiotic (water content, temperature, texture etc.) and biotic (soil microbial communities, microbial enzymes etc.). This study suggested that addition of olive litter and leaves enhanced soil organic carbon mineralization. In conclusion, olive leaves and litter are beneficial for the soil microbial communities.

5. ACKNOWLEDGEMENTS

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