Priming effects of leaves of *Laurus nobilis* L. and 1,8-cineole on carbon mineralization

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ABSTRACT

Plant secondary compounds can have stimulating effect on C cycling and change its rate in soils. We examined how leaves of bay laurel (*Laurus nobilis* L.; Lauraceae) and 1,8-cineole (CIN), one of its constituents, affect soil C mineralization and its rate. Leaves and soil samples of bay laurel were taken from Cukurova University Campus (Adana, Turkey) growing naturally under Mediterranean climate conditions. Leaves and CIN were considered as the two forms of organic C sources. After determining the level of 1,8-cineole in leaves by gas chromatography-mass spectrometry, soils were mixed with powdered leaves and 1,8-cineole based on their C contents at same and half doses of soil organic C level. Carbon mineralization of all soils was determined over 54 d (28 °C, 80% field capacity). While 1,8-cineole was found as a major constituent of leaves (65% of essential oil), all doses of leaves and CIN increased soil microbial activity. There were significant differences for C mineralization rate between control and all applications ($P < 0.05$). High C levels of all treatments decreased C mineralization rate compared to control soils. In summary, all treatments stimulated C mineralization and it is possible to conclude that soil microorganisms adapted to use CIN as an energy source.

Key words: Bay laurel, carbon mineralization, 1,8-cineole, essential oil, GC-MS.

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INTRODUCTION

Soil organic matter (SOM) mineralization plays a key role in global climate change and it is a popular topic in recent years as soil microbial community is one of the most important factors that regulate this process (Wang et al., 2013). Main inputs of organic matter stocks in soil are primarily leaves and their constituents while the efflux of carbon dioxide (CO_2) from soil through atmosphere are outputs (Davidson and Janssens, 2006). Addition of C sources can change the turnover of SOM in a short period (priming effect) and this can be measured with changes in CO_2 efflux rates (Kuzyakov et al., 2000).

Carbon mineralization is an effective method for quantifying the effects of various organic sources on soil functions (Haney et al., 2004). It is extensively measured by tracking CO_2 fluxes from field-moist soils which are wetted to 50%-80% field capacity and afterwards incubated in the laboratory for various periods of time (Aka Sagliker et al., 2014). Knowing of how soil organic C (SOC) mineralization responds to external C sources like leaves or litters is essential to better understand whether soils will react to climate change and increase the release of CO_2 to the atmosphere. On the other hand, environmental conditions like temperature, moisture and organic matter quality (e.g. C/N ratio) have been considered to be the special factors of SOC mineralization, but the extensive role of biotic factors (e.g. microbial activity) are now widespread recognized (Strickland et al., 2009). Gomoryova (2004) clearly informed that respiration rate of soil microflora is an indicator of the microbial activity that regulates both soil nutrient dynamics and organic matter turnover.

Various hydrocarbons, isoprenes and monoterpenes are belong to a large molecule group called volatile organic compounds (VOC). Among the VOCs, monoterpenes are important because of loading the atmosphere with emission rates of about 127 Tg C yr^{-1}, which is approximately 9% of total VOC emission rate (Marmulla and Harder, 2014). Below toxic concentrations, soil microorganisms can use monoterpenes as a C source and this compounds can stimulate soil respiration (Vokou et al., 2002; Marmulla and Harder, 2014).

Monoterpenes are main constituents of the essential oils of aromatic plants (Misra et al., 1996). 1,8-Cineole (CIN) has been found main component of essential oil of *Laurus nobilis* L., an aromatic plant of Mediterranean region (Sangun et al., 2007; Yilmaz et al., 2013). Bay laurel is a perennial shrub with aromatic evergreen leaves and Turkey is an important exporter country

(18th place) of aromatic plants in the world (Lange, 2002). In 2002, Turkey exported 4869 tons of bay leaves for a return of USD 7.7 million (Ozek, 2012).

CIN is one of the most valuable and important monoterpenes because its antimicrobial (Van Vuuren and Viljoen, 2007) and antifungal (Morcia et al., 2012) effects. There are many reports on antimicrobial effects of both leaves of *L. nobilis* and 1,8-cineole (Van Vuuren and Viljoen, 2007). While effects of monoterpenes on nutrient cycling were widespread studied (White, 1994; Marmulla and Harder, 2014) as far as our knowledge about their effects on C mineralization is limited.

The objective of current study is to provide an insight about the effects of leaves of *Laurus nobilis* and 1,8-cineole on C mineralization and to determine the amounts of 1,8-cineole in leaves by GC-MS. This study evaluates how addition of these two forms of C sources influence the response of C mineralization and make a comparison with each other.

MATERIALS AND METHODS

Soils and leaves of bay laurel (*Laurus nobilis* L.) have been taken from Cukurova University Campus, Adana (37°3'26" N, 35°21'19" E), Turkey, in January 2010. Semi-arid Mediterranean climate conditions have been characterized in Adana (mean annual precipitation: 659 mm; mean annual temperature: 19.1 °C for 35 yr) (Kum and Çelik, 2014).

Both soils and leaves were air-dried. While soils were sieved through a 2 mm mesh sieve and leaves have been powdered before analysis. Soil texture was determined by Bouyoucos hydrometer (Beretta et al., 2014), field capacity (%) by 1/3 atmospheric pressure with a vacuum pump (Kacar, 2012), pH by a 1:2.5 soil-water suspension with pH-meter (inoLab pH/Cond 720, WTW GmbH, Weilheim, Germany) (Kacar, 2012), $CaCO_3$ content (%) by a Scheibler calcimeter (Kacar, 2012). Organic C and total N contents of soils and leaves (%) were determined by modified Walkley and Black method and Kjeldahl method, respectively (Saez-Plaza et al., 2013; Sato et al., 2014).

Some physical and chemical properties of soils and leaves

Soils were sandy loam and slightly alkali. Field capacity of soils were 53.42% while $CaCO_3$ were 5.73%. Carbon amounts of soils and leaves were found 3.71% and 49.71%, N contents were 0.57% and 1.88%, respectively (Table 1).

Table 1. Some physical and chemical properties of the soils and leaves of *Laurus nobilis* (mean ± SE; n = 3).

Samples	Characteristics	
Soils	Clay, %	14.58 ± 0.00
	Silt, %	8.21 ± 0.00
	Sand, %	77.21 ± 0.00
	Texture type	Sandy loam
	pH	7.52 ± 0.05
	Field capacity, %	53.42 ± 0.00
	$CaCO_3$, %	5.73 ± 0.16
	C, %	3.71 ± 0.08
	N, %	0.57 ± 0.03
	C/N	6.49 ± 0.37
Leaves	C, %	49.71 ± 6.34
	N, %	1.88 ± 0.00

Hydrodistillation and GC–MS analysis

A sample of 20 g of powdered *L. nobilis* leaves was submitted for 3 h to water distillation using a Clevenger type apparatus. After obtaining essential oil of leaves by distillation, quantification of 1,8-cineole in essential oil were carried out by using GC-MS analyzer (Triplus Trace GC Ultra ISQ, Thermo Scientific, Waltham, Massachusetts, USA). A sample (10 μL) obtained from hydrodistillation was injected and chromatographic separations were accomplished with a TR-MS-5 capillary column (5% phenyl-95% dimethylpolysiloxane, 0.25 mm id × 60 m, film thickness 0.25 μm). The column temperature was programmed as following sequence respectively: at 50 °C for 1 min, 160 °C, 3 °C min^{-1} for 3 min, and 250 °C at 5 °C min^{-1} for 10 min. The injection port temperature was 240 °C and the applied ionization voltage was 70 eV. Identification of CIN was performed by matching with GC-MS results of National Institute of Standards and Technology (NIST, Gaithersburg, Maryland, USA) mass spectral library data. The amount of CIN in leaves was determined using internal standard calibration method in GC-MS and toluene was used as internal standard (Giray et al., 2008). 1,8-Cineole was found as a major active component of leaves by GC-MS analysis. Volume of obtained volatile oil from 20 g powdered bay leaves was found 0.15 mL while the amount of CIN were 6.5 mg 10 μL^{-1} oil (65%).

Experimental design and statistical analysis

Powdered leaves and CIN ($C_{10}H_{18}O$, M: 154.25 g mol^{-1}; Merck, Darmstadt, Germany) based on their C contents were separately mixed with soils at the same (L_S, C_S) and half (L_H, C_H) doses of control soil organic C level (3.71%). Both organic C levels of L_S and C_S were 7.42% after mixing 100 g soils with 7.46 g powdered leaves (L_S) and 4.76 g pure CIN (C_S) while C contents of L_H and C_H were 5.57% by mixing half amount of L_S and C_S. Control was untreated soil to determine the effects of both C sources on C mineralization.

Soil samples (100 g) were placed in 750 mL incubation vessels and the final moisture contents of both soils were adjusted to 80% of their own field capacity before incubation at 28 °C over 54 d (Jarvis et al., 2007). The CO_2 produced from microbial activity was absorbed periodically in 40 mL saturated $Ba(OH)_2$ solution in small tubes, which were placed on the top of the soil in incubation vessels. Every 3 d CO_2 produced by microbial respiration was measured by titration with oxalic acid in these closed vessels (Cheng et

al., 2013). Cumulative C mineralization (mg $C(CO_2)$/100 g soil) was calculated by summing up all 3 d CO_2 until end of incubation period while its rate was calculated by dividing cumulative mineralized C by its soil organic C of control and all treatments. Also, C mineralization rates were calculated at day 30, day 30-54 and day 54 to find differences in all treatments and rate of control soil were taken as 100% to find and compare increase and decrease percentage of all treatments.

Tukey honestly significant difference (HSD) test was performed to determine the differences in C mineralization over incubation time among the treatments (Kleinbaum et al., 1998). Results are given in figures as mean values ± standard errors of three replicates. Differences between the data were declared as significant at the $P < 0.05$. All statistical analysis were carried out using SPSS v.22 (IBM, Armonk, New York, USA).

RESULTS AND DISCUSSION

Aka and Darici (2005) and Zengin et al. (2008) found slightly similar results of soil C, N, texture and pH, but our field capacity result was found higher than these studies. This difference may have come from different sampling sites in campus.

We found that CIN was a major component of *L. nobilis* leaves (65% of essential oil) by GC-MS analysis that correlates with the findings of Sangun et al. (2007) (50%), Yilmaz et al. (2013) (51.8%), and Da Silveira et al. (2014) (39.81%). Geographic origin, seasonal and genetic variations may affect composition of essential oils (Ozcan and Chalchat, 2005; Sangun et al., 2007).

Cumulative C mineralization of control, C_H, C_S, L_H, and L_S, were 33.31, 36.55, 43.91, 45.53 and 44.41 mg $C(CO_2)$ 100 g^{-1} soil, respectively (Figure 1). Comparing to control soils, L_S dose increased C mineralization through all incubation period from the beginning, while it was increased after day 9 at L_H dose, after day 18 at C_S dose and after day 30 at C_H (Figure 1). At the end of the incubation period, there were found significant differences in C mineralization between control and other dose treatments except C_H ($P < 0.05$). While there was significant difference between C_H and L_H doses ($P < 0.05$), nonsignificant difference has been found between L_S and C_S doses ($P = 1.00$). Although there are some reports on high antimicrobial activity of CIN and leaves of *Laurus nobilis* (Sato et al., 2007; Van Vuuren and Viljoen, 2007; Yilmaz et al., 2013). Our results showed that added all doses of leaves and CIN have stimulated microbial respiration except of C_H dose (Figure 1). However, C_H dose have stimulated the respiration slightly compared to control after 33 d. This result correlates with results of Vokou et al. (2002) who found mixing monoterpenes with soils would increase microbial activity. In addition, Adamczyk et al. (2011) reported that terpenes can stimulate C mineralization and microbial biomass because soil microbes can use these terpenes as a C source. Our results clearly showed that microorganisms can use CIN as a C source but also could use leaves much more efficiently than CIN because of the possibility of lower amounts of CIN in leaves.

Fontaine et al. (2003) claimed that addition of fresh organic matter input to soil, many specialized microorganisms, classified as r-strategists, only decompose fresh organic matter (FOM) are and there are K-strategists which are continuously active and they decompose only soil organic matter (SOM). Same authors indicated that even if large amount of energy and nutrients, K-strategists may not have enough time to assimilate these because they grow too slowly compared to r-strategists. This revision contributes this study because it is possible to say that stimulation of C mineralization by addition of leaves made by r-strategists compared to control. Carbon mineralization was first decreased then increased by addition of CIN after 21 d compared to control. Thus, CIN may have prevented the usage of SOM for first 21 d and then may be recognized as an energy source by both K and r- strategists after 21 d. However, stimulation of C mineralization by C_S dose more than C_H dose can be explained with high C content of C_S which is sufficient to provide growth of microorganisms more than C_H.

Compared to control soil, C mineralization rate was decreased by 33% at C_H, 43% at L_H and 27% at L_S but increased by 2% after 30 d. While there were significant differences between control and all CIN and leaf treatments), it was found significant differences between L_H and all CIN treatments ($P < 0.05$, Figure 2). After subtraction of 30 d of mineralization rate, ratio of between days 30 and 54 was calculated and it was found that rate of mineralization was decreased by 21% at C_H, 25% at C_S and 20% at L_H, and 40% at L_S compared to control soil (Figure 3). While there were

Figure 1. Cumulative carbon mineralized in the control, soils mixed with 1,8-cineole and leaves based on their carbon contents at the same and half doses of organic carbon level of control soils (mean ± SE, n = 3).

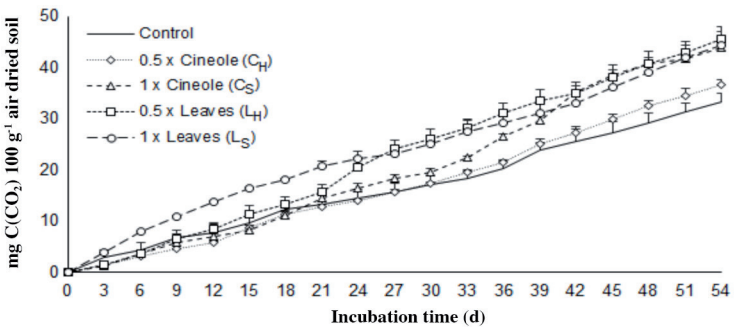
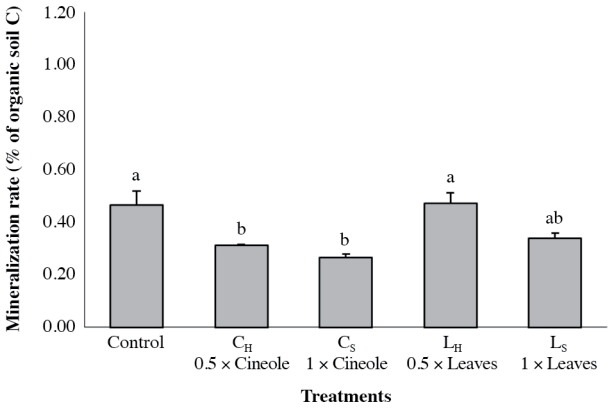


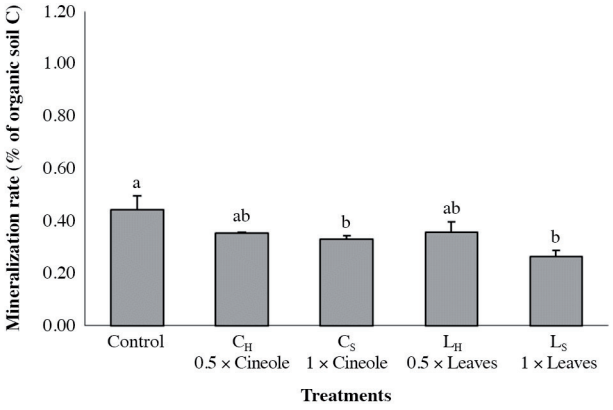
Figure 2. Rate of mineralization of organic carbon in the control and all treatments (mean ± SE, n = 3) at 28 °C for first 30 d.



Different letters denote significant differences among control and applications ($P < 0.05$).
Treatments: Powdered leaves (L) and 1,8 cineole (C) were separately mixed with soils at the same (L_S, C_S) and half (L_H, C_H) doses of control soil organic C level.

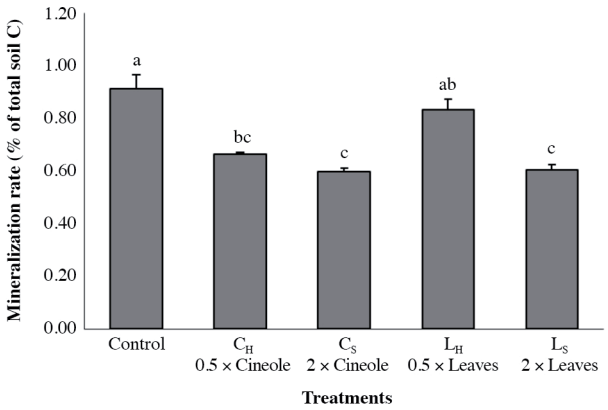
significant differences between control and both C_S ($P = 0.019$) and L_S ($P = 0.001$), nonsignificant differences were found among control and both C_H and L_H, separately ($P > 0.05$). After 54 d, rate of mineralization was decreased by 27% at C_H, 35% at C_S, and 9% at L_H and 34% at L_S compared to control soil (Figure 4). While there were significant differences between control and all treatments ($P < 0.05$) except L_H ($P = 0.691$), significant differences were found among L_H and both L_S and C_S ($P < 0.05$). Aka and Darici (2005) found that mixing Aleppo pine (*Pinus halepensis* Mill.) and Kermes oak (*Ceratonia siliqua* L.) leaves with Campus soils have increased both their C mineralization and its rate as our study have similar results with difference that decrease of ratio of C mineralization by all treatments.

Figure 3. Rate of mineralization of organic carbon in the control and all treatments (mean ± SE, n = 3) at 28 °C between days 30 and 54.



Different letters denote significant differences among control and applications ($P < 0.05$).
Treatments: Powdered leaves (L) and 1,8 cineole (C) were separately mixed with soils at the same (L_S, C_S) and half (L_H, C_H) doses of control soil organic C level.

Figure 4. Rate of mineralization of organic carbon in the control and all treatments (mean ± SE, n = 3) at 28 °C during the incubation time (54 d).



Different letters denote significant differences among control and applications ($P < 0.05$).
Treatments: Powdered leaves (L) and 1,8 cineole (C) were separately mixed with soils at the same (L_S, C_S) and half (L_H, C_H) doses of control soil organic C level.

This can be explained with the increase in organic C level of treatments by addition of leaves.

While it was observed that there was a clear and stable soil microbial activity there is a strong presence of ecological balance and new introduced compounds were decomposed by tolerating at certain doses in sampling sites. Despite of very low CIN amounts of leaves, it is an interesting point that C mineralization rate was decreased at L_S dose. In this case, it is not possible to say that CIN inhibited soil microbial activity. As C mineralization rate was higher at L_H dose but lower at L_S dose than control. Because of the amount of decomposing leaves, this result may be explained with insufficient oxygen level. On the other hand, soil microorganisms at CIN doses might have avoided negative effects of CIN with their selective absorption and used it as an energy source.

Based on these results, it is appeared that plant constituents should be considered as a whole. In conclusion, evaluating main constituents in plant parts separately will be better approach.

CONCLUSIONS

Mechanisms of priming effects are made of complex and long processes in nature. Aim of this study was to give an insight about decomposition both 1,8-cineole (CIN) and bay leaves in soil thorough C mineralization and to contribute knowledge about priming effect and understanding of itself. It is suggested that determining microbial populations (bacterial, fungi count, etc.) and extracellular enzyme activities like cellulase and xylanese during C mineralization and extending mineralization process more than 2-mo with same and different doses of CIN or different constituents of leaves of *Laurus nobilis*. Also because of global warming, temperature and moisture of soils should be changed to get better results.

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