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Effect of α-Pinene on the Fate of Microorganisms and Soil Organic Carbon Content in Eucalyptus and Corymbia Plantation Forests

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Abstract

Alpha-Pinene is secreted by some tree species including *Eucalyptus grandis* and *Corymbia torelliana*. This monoterpene is known to affect microorganisms and therefore it could affect soil microbial processes including organic matter (OM) decomposition. To study the effect of α-Pinene on soil microorganisms and soil organic carbon (SOC), the soils taken from 7-years-old *E. grandis* and *C. torelliana* plantations in Passara, Sri Lanka were analysed for total organic carbon (TOC), microbial biomass carbon (MBC), bacterial and fungal populations, and α-Pinene content. As the control, the soils of an adjacent grassland were analyzed. The results revealed that *E. grandis* plantation had the highest TOC, MBC, bacterial population and α-Pinene content while the lowest of these parameters were observed in grassland soil including the highest fungal population. Interestingly a significant positive correlation was observed between α-Pinene content and TOC in the soils of the study sites ($P = 0.000$). The results also indicated a significant positive correlation between α-Pinene content and, the bacterial population while the fungal population showed a significant negative relationship ($P < 0.05$). When the fungal population is retarded possibly due to the activity of α-Pinene, the decomposition process could also slow down resulting in more TOC in plantation soils. Therefore, we could mention that α-Pinene content in soil could affect soil microorganisms and their processes and therefore, it can be considered as an important factor that determines carbon sequestration potential in Eucalypt plantation forest soils.

Keywords: α-Pinene, *Corymbia torelliana.*, *Eucalyptus grandis,* bacteria, fungi, soil organic carbon (SOC)

1. Introduction

Due to fast growth and high adaptability, Eucalyptus is extensively planted covering more than 20 million hectares of land in the world to obtain timber, fuel wood, pulp for paper, etc. [1]. Also, some of these plantations are grown to obtain essential oils for medicinal purposes and perfume industry in addition to preventing water and wind erosion [2, 3]. Other than these uses recent studies show that plantations such as Eucalypts could be a good sink for carbon (C) and thus proposed to be used in reducing the level of atmospheric carbon dioxide (CO_2) [4-6].

At present, steadily increasing atmospheric CO_2 concentration has created serious environmental issues like global warming and climate change [7]. Finding mitigation options to manage CO_2 levels in the atmosphere has become a major challenge at present and therefore, the use of these plantation forests for this need could be a long-term profitable solution. Eucalypt plantations sequester C in both above-ground components and in the soil as SOC. From these, soil C is very important because they comprise almost two-thirds of the C stored in forest ecosystems [8]. Eucalypt plantations are known to store a high amount of C in soil [6]. However, the information on mechanisms of soil C sequestration in Eucalypt soil is still lacking and identification and understanding of these mechanisms are also important in promoting C sequestration in these plantations.

Soil receives C from plants, plant residues and other organic solids which are stored or reserved in the soil as part of the SOM [9]. However, this stored C is emitted back to the atmosphere via SOM decomposition, mainly due to the activities of soil microorganisms [10]. Bacteria and fungi play a major role in the SOM decomposition process [11] and if their activities could be controlled, the SOM decomposition rate can be slowed down. Many factors could affect soil microbial activities in soil including temperature, water availability and organic and inorganic chemicals that are available in soil [12,13].

Eucalypt trees contain many volatile organic chemicals such as monoterpenes which can affect the activities of microorganisms [14]. α-Pinene is one such compound that is available in greater concentrations, especially under *Eucalyptus grandis* and *Corymbia torelliana* forest plantations [15-17]. Further, α-Pinene has shown antimicrobial activities against many microorganisms. For example, some studies reported [18-19] effect of α-Pinene against many bacterial and fungal species. Therefore, there is a possibility that soil α-Pinene can act against soil microbiota and interrupt normal soil functions including SOM decomposition processes which in return influence soil carbon (C) sequestration [20]. However, information on how α-Pinene affects soil microbial biota, especially bacteria and fungi, and how it affects SOC are still lacking. Therefore, the present study was designed to investigate the effect of α-Pinene on the fate of soil microorganisms and SOC content in Eucalyptus and Corymbia plantation forests. Two 7-years-old *Eucalyptus grandis* and *Corymbia torelliana* plantations were selected for the study and our primary objective was to study the effect of α-Pinene on soil microorganisms and SOC Here we hypothesized that all the other parameters other than plant species in Eucalyptus, and Corymbia plantations and grassland are same.

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2 Materials and methods

1.1 Study Area

This study was carried out in *E. grandis, C. torelliana* forest plantations and in an adjacent grassland located in Passara, Badulla district, Sri Lanka (7° 5' N - 9° 4' N and 81° 10' E - 83° 15' E). The climate is tropical and monsoonal and the region has a mean annual rainfall of 2,245 mm which is received between October and February via the North Eastern monsoon. The mean annual temperature of the area is approximately 19 - 23 °C and the elevation is 868 m asl. The soil type is typical Haplohumults fine loamy, acidic, non-calcareous and isohyperthermic soils [21].

1.2 Site Description

E. grandis, C. torelliana forest plantations and the grassland were in the same agroecological zone (IM 2). All these selected sites were located within a radius of 5.5 km and spread in natural rugged topography with a moderate slope (<4-6%).

1.3 Eucalyptus Plantations

Before establishing Eucalypt plantations, the area was covered with Montane grasslands mainly with *Cymbopogon nardus* (L.) Rendle. Substantial site preparation and removal of shrubs and weeds before establishing forest plantations had been carried out in these sites. About 50 g plant^{-1} of fertilizer (N:P: K) = 9:11:9) have been added at the time of plantation establishment.

Three 20 m x 20 m plots were established in each plantation and grassland. Each plot in plantations contained 32-40 trees and the plots were demarcated using a Brunton altimeter, meter tapes and strings. Slope and topographic variation were considered to be the minimum among the plots and field sites. Each plot was divided into four interior subplots (10 m x 10 m) for better sampling.

1.4 Soil Sampling

Soil samples were taken from 3 randomly selected points in each subplot up to 30 cm using a hand auger (50 mm diameter). The litter layer was removed before sampling and the samples taken from an interior subplot were pooled into one composite sample. Collected soil samples were placed in properly labeled air-tight polyethylene bags and transported to the laboratory.

In the laboratory, stones and roots were removed using a 2 mm sieve. Soil pH, soil moisture content and microbial biomass carbon (MBC) in fresh soil were analyzed using standard protocols. Before further analysis, the leftover soils were air dried and ground into a powder measuring less than 0.15 mm using an M20, IKA, or WERKE® grinder.

1.5 Soil Analysis

1.5.1 Enumeration of bacteria and fungi in soil

Quantification of soil bacteria and fungi was carried out using plate counts of culturable microbe's method with nutrient agar (NA) and potato dextrose agar (PDA) and PDA, respectively [22]. Here 1 g of soil was added to a 10mL of sterilized distilled water in a sterilized test tube. The content was mixed well and from it, 1 mL was taken and mixed with 9 mL of sterilized distilled water in another sterilized test tube. If necessary, dilution series was prepared using the initial sample. Then using a sterile, pipette 1 mL from each dilution was spread on the nutrient agar and PDA plates. From each sample, three replicate plates were prepared. The cultures were incubated at room temperature for 24 hrs for bacteria and 72 hrs for fungi.

1.5.2 Determination of MBC and TOC

Microbial biomass c (MBC) was determined by using the chloroform fumigation and extraction method (Vance *et al.*, 1987). After fumigation, MBC was extracted using 0.5 M K_2SO_4 and quantified by using a CHN analyzer (Elemental z analyzer, Perkin-Elmer 2400 series II). Total organic carbon (TOC) content in soils was determined by the dry combustion method using a CHN analyzer (Perkin-Elmer 2400 series II).

1.5.3 Determination of α-Pinene content in soil

Determination of α-Pinene concentration in soils was conducted following the methodology described by Wilt *et al*. [24]. The soils were ground up (up to around 1 mm) in a clean mortar and pestle before being ready for analysis. Ten-gram aliquots of ground soil were placed in 25 mL test tubes and extracted with 10 mL of petroleum ether. Samples were subjected to 60 s of vigorous shaking on a Pulser Vortex Test tube Mixer. The solvent and sample were allowed to equilibrate for 48 h at room temperature. The extracts were then filtered through Whatman No. 01 filter paper and washed with 100 mL petroleum ether. The resulting extracts were concentrated to 0.5 mL, under nitrogen in a calibrated centrifuge tube. Each sample was then analyzed by injection of extract into a Shimadzu GC 2010 gas chromatograph using a 0.25 μm F.I.D.X 30 m RTX wax capillary column.

The oven temperature was programmed from 60 to 225 °C at the rate of 5 °C min^{-1}.

1.6 Statistical Analysis

Minitab (19.2 0) Software was used for all statistical analyses. The means and associated standard deviations of the proportion of soil bacterial and fungal counts, MBC, TOC, and α-Pinene were calculated. One-way analysis of variance (ANOVA) was used to test the effect of vegetation type on the proportion of the number of studied variables. Means were compared using the Tukey test at P=0.05. Regression analyses were performed between soil α- Pinene content and all other variables studied. Pearson correlation coefficient was used to study the relationships between the variables studied.

3 Results and discussion

There was a significant (P<0.05) difference in pH values of the studied sites (Table 01). Accordingly, the highest pH value was observed in the grassland soil while the lowest was observed in *E. grandis* forest plantation soil. Acidification of soils in Eucalyptus plantations could be instigated by natural processes associated with the decomposition of organic matter in organic horizons [25], mainly because acidic compounds such as phenolic compounds exist in Eucalypt litter [26].

Table 1: Variation of pH and moisture content (MC) in *Eucalyptus grandis*, *Corymbia torelliana* plantations and grassland soils. The least significant (alpha = 0.05) differences between any two means are based on Tukey's pairwise comparisons, and different letters denote the significant differences between vegetation types.

Site	pH	MC (%)
Eucalyptus grandis	5.12 [b]	40.1 [b]
Corymbia torelliana	5.42 [ab]	49.9 [a]
Grassland	5.68 [a]	27.6 [c]

Moisture content (MC%) also varied significantly (P<0.05) in the plantations and grassland soils (Table 01). The highest MC was observed in the *C. torelliana* plantation while the lowest was observed in the grassland. According to Kasischke and Johnstone [27], MC varies as a function of the thickness of the litter layer. The litter layer in plantation forests is relatively higher than the litter in grassland. Therefore, we can assume that the litter layer may have affected the significantly higher MC in plantation forests. Other physicochemical properties may have affected higher MC in *C. torelliana* plantation compared to *E. grandis* plantation; however, this should be further studied.

According to the results, soil bacterial population, fungal population, MBC, TOC, and α- Pinene (%) were significantly (P<0.05) affected by the type of vegetation.

Bacterial and fungal populations significantly (P<0.05) changed among the three vegetation types (Table 2). The highest bacterial population was found in *E. grandis* plantation forest (2.7 x 10^5 CFU) while the lowest was found in the grassland soil (3.9 x 10^4 CFU) (Table 2). The greater bacterial population in *E. grandis* over *C. torelliana* may be attributed to the soil-environmental conditions where in particular, the soil pH plays a key role. Cho *et al*. [28] found that soil bacterial diversity, evenness, and richness are usually higher in lower pH values.

The highest fungal population was detected in the grassland soil (2.0 x 10^3 CFU) while the lowest was recorded in *E. grandis* (5.3 x 10^2 CFU) (Table 2). However, according to Rousk [29], soil fungi favor lower soil pH values, which contradicts our results. But some studies have mentioned that α-Pinene could inhibit the growth of certain fungi [30, 31]. Therefore, for the variation in fungal population observed among treatments, α-Pinene concentration in the soil might have been affected.

Table 02: Variation of bacteria and fungi populations, and Microbial biomass carbon (MBC) in *Eucalyptus grandis*, *Corymbia torelliana* plantations and in grassland soils. The least significant (alpha = 0.05) differences between any two means are based on Tukey's pairwise comparisons and different letters denote the significant differences between vegetation types.

Site	Bacterial population (CFU/mL)	Fungal population (CFU/mL)	MBC
Eucalyptus grandis	2.7 x 10^5 [a]	2.8 x 10^2 [b]	12.9 ± 4.0 [a]
Corymbia torelliana	5.2 x 10^4 [b]	5.3 x 10^2 [b]	7.09 ± 2.6 [b]
Grassland	3.9 x 10^4 [c]	2.0 x 10^3 [a]	3.08 ±1.6 [c]

Different vegetation types significantly (P<0.05) affected the TOC content. The highest TOC was observed in *E. grandis* plantation forest (21.94 g kg^{-1}) while the lowest was observed in the grassland soil (1.69 g kg^{-1}, Fig. 1). Since the biomass production of *E. grandis* is high [32] the observed difference in TOC content in *E. grandis* and *C. torelliana* could be related to the biomass production of each and the amount of organic matter added thereafter into the soil.

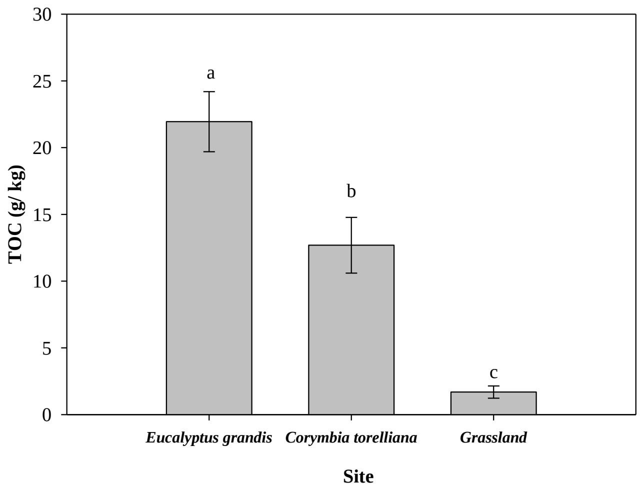

Fig. 1. Variation of Total Organic Carbon (TOC) in *Eucalyptus grandis*, *Corymbia torelliana* plantations and grassland soils. The least significant (alpha = 0.05) differences between any two means are based on Tukey's pairwise comparisons, and different letters denote the significant differences between vegetation types.

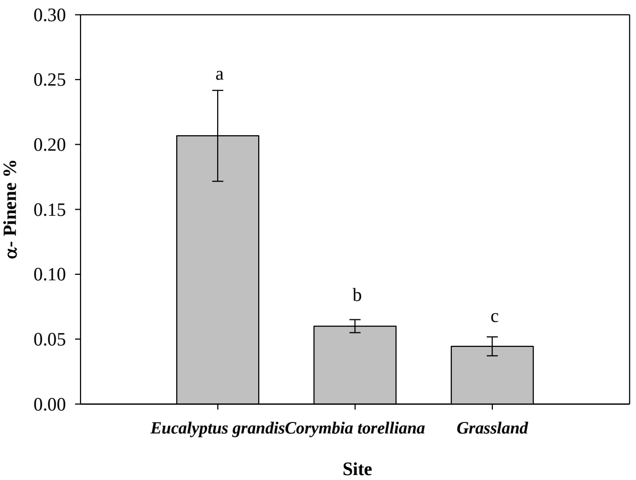

Fig. 2. Variation of α- Pinene content in soils of *Eucalyptus grandis, Corymbia torelliana* plantations and grassland. The least significant (alpha = 0.05) differences between any two means are based on Tukey's pairwise comparisons, and different letters denote the significant differences between vegetation types.

The amount of α-Pinene present in the three soils was significantly (P<0.05) different from each other (Fig. 2) and the highest (0.21%) and the lowest (0.04%) percentages of α- Pinene were recorded against *E. grandis* and grassland, respectively. Production of α- Pinene seems to have a clear positive relationship with TOC which means it is again related to the biomass production of Eucalyptus plants. Greater the biomass production, higher the α-Pinene production and TOC in soil (Fig. 1 and 2; Pearson correlation = 0.841, P<0.05).

A significant positive correlation was observed between TOC and bacterial populations in the studied sites (Pearson correlation = 0.537, P<0.05). Conversely, the fungi population acted over bacteria. There was a negative correlation between the fungal population in the soils with TOC (Pearson correlation = -0.670, P = 0.000). Significantly (P<0.05) the highest population of fungi was found in grassland while the lowest was observed in *E. grandis* forest plantation. The results also showed that the highest amount of MBC was from *E. grandis* plantation forest soils and the lowest was from the grassland soils (Table 1). A significant positive correlation was found between MBC and TOC in all tested soils (Pearson correlation = 0.797, P=0.000). For *E. grandis* plantation forest, α-Pinene content was 0.20%, which was significantly (P<0.05) higher compared to values obtained for *C. torelliana* plantation and grassland soils (0.06 and 0.04%, respectively) (Fig. 2).

Based on the correlation analysis, the regression lines in Fig. 3 describe the relationships between α-Pinene content and TOC, bacterial and fungal population and MBC in the studied sites. A significant positive correlation was observed between α-Pinene and TOC (Pearson correlation = 0.841, P=0.000). The results also indicated a significant positive correlation between α-Pinene content and bacterial population and MBC (Pearson correlation = 0.625 and 0.710 respectively, P<0.05) (Fig. 3, b and d). However, the fungal population showed a significantly negative relationship with α-Pinene content in soil (Pearson correlation = -0.483, P<0.05) (Fig. 3, c).

α-Pinene is a monoterpene that can be found in high concentrations in *E. grandis* and *C. toreliana* plant parts [15] With litter and from many other sources, α-Pinene mixes with soil and in turn affects soil processes [33]. This monoterpene is also known to show antimicrobial activity toward many microorganisms [18,34] but some studies show that they promote the growth of some soil microorganisms as well [35]. Soil microorganisms are an important component in the soil as they play a major role in transforming SOM via the degradation process into different SOC pools for both short and term soil C sequestration.

In the present study, the highest amount of TOC was found in *E. grandis* plantation forest. Many factors can influence the TOC content in plantation soils such as soil nutrients, stand age, climate, soil texture, bulk density, moisture content, pH and microorganisms [36-38]. Since the studied *C. torelliana* plantation and *E. grandis* plantations are of the same age and in the same area, it is assumed that all the other conditions that affect soil are similar. However, our study results revealed that α-Pinene content in soil was higher in *E. grandis* plantation compared to *C. torelliana* plantation and grassland. In contrast, Coffi *et al*. [15] reported a higher amount of α-Pinene in *C. torelliana* compared to *E. grandis* soil.

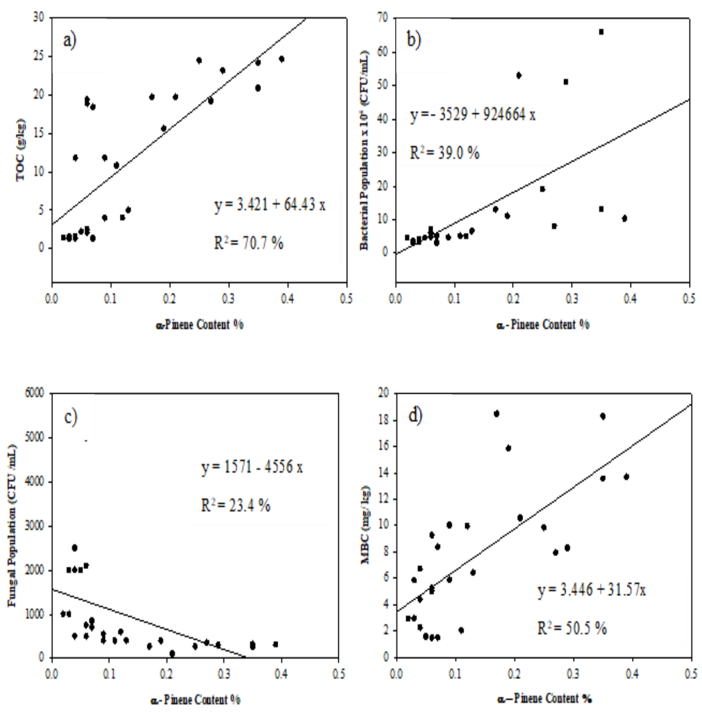

Fig. 3. Correlations between α-Pinene content and a) Total Organic Carbon (TOC) b) Bacterial population c) Fungal population d) Microbial Biomass Carbon (MBC) content in *Eucalyptus grandis*, *Eucalyptus torelliana* and grassland soils.

Many factors influence on production and emission of monoterpenes in trees including *Eucalyptus* and *Corymbia* spp. According to Yokouchi and Ambe [39], temperature, light conditions and saturated vapor pressure in leaves directly influence the emission of monoterpenes from trees. Moreover, the rate of monoterpene biosynthesis is affected by $^{14}CO_2$ incorporation in leaves [40]. Overall, all these given conditions and factors could have affected on the higher amount of α- Pinene content in *E. grandis* plantation over *C. torelliana*.

Besides, α-Pinene content in the soil seems directly affect on fungal population compared to the bacterial population in the studied sites. Bacteria populated better in the presence of α-Pinene whereas the opposite was reported for fungi (Table 1, Fig. 2). These results are in accordance with the findings of Adamczyk [41] and Kleinheinz *et al*. [42]. Some bacteria utilize α-Pinene as their C and energy source. Bacteria such as *Pseudomonas fluorescens* and *Alcaligenes xylosoxidans* are found to utilize α-Pinene as their C and energy source [42]. Yoo and Day [43] also confirm that *Pseudomonas* spp. use α-Pinene as a C source in the soil. This could be the reason for observing a high bacterial population in *E. grandis* plantation which had a high amount of α-Pinene content in the soil. Thus, it resulted a positive correlation between soil bacteria and α-Pinene content in the soil. Nevertheless, this was different for the fungi population suggesting that α-Pinene could have acted as a toxin to fungi [41]. Ludley *et al*. [44] have found that vapors of α-Pinene upsurge colonization of tree roots by ectomycorrhizal fungi and reduce the respiration rate of some saprotrophic fungi. This compound mainly acts against the cytoplasmic membrane of fungi and destroys cellular integrity by inhibiting the respiration process. Also, it inactivates the synthesis of intracellular and extracellular enzymes [45]. This antifungal activity of α- Pinene may have been the reason for lower fungal populations in *E. grandis* plantations which had a high amount of α-Pinene compared to two other sites.

MBC also showed a significant positive correlation with α-Pinene content in the soil. This can be explained rather by bacterial growth but not by fungal growth. Similar results are reported by Adamczyk [41] who has studied the effect of terpenes on microbial biomass and, C and N cycling processes in boreal forest soil in Finland. Accordingly, higher MBC in the soil is represented by bacteria. TOC is a good determinant of MBC content [46,47].This was confirmed with our results as well by displaying a significant positive correlation between TOC and MBC (Pearson correlation = 0.797, *P*=0.000). Even though MBC represents 1-3% of TOC, microorganisms play a major role in soil structural development [48,49].

Several factors might have increased TOC in *E. grandis* plantation forest compared to the two other studied sites. However, it is obvious from the present study that α-Pinene plays a key role in this regard. When the α-Pinene concentration was high the bacterial population increased, but the opposite was observed for the fungal population. It is known that fungi are the principal agents of decomposition and bacteria usually play relatively a minor role [50]. This is confirmed by Pascoal and Cássio [51] as well. Further, Glassman *et al*. [52] have shown that bacterial communities could be functionally unessential than fungi with regards to SOM decomposition. Although there was a higher MBC, which is represented by more bacteria than fungi in *E. grandis* plantation, decomposition rates of organic C in soils were lower compared to *C. torelliana* and grassland. With a comparatively higher amount of fungal population in *C. torelliana* and grassland soils, it could not sequester more C due to their higher decomposition rates.

4. Conclusions

α-Pinene, the monoterpene, which is highly available in *E. grandis* and *C. torelliana* plant parts, can affect soil processes. TOC content in *E. grandis* plantation forest soil was significantly higher than that of the *C. torelliana* and grassland soils. As per the findings, α-Pinene content in soil was positively correlated to TOC, bacterial population, and MBC in soils but it was negatively correlated to soil fungal population. Thus, it is apparent that the fungal population is significantly affected by α-Pinene content in soil and thereby possibly slowing down the decomposition rate in *E. grandis* plantation forests increasing their TOC content in the soil. Therefore, soil α-Pinene content could be considered as an important factor that determines C sequestration potential in Eucalyptus plantation forest soils.

Conflicts of Interest

No conflicts of interest are disclosed by the authors.

Acknowledgments

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