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Effects of Different Incubation Periods on Microbial Biomass Carbon (MBC) in Two Soil Series

D.S. Karam[1,*], M.N.C. Jamil[1], N. Jaafar[1], K.S. Rajoo[1], A. Arifin[2]

[1]*Department of Land Management, Faculty of Agriculture, University Putra Malaysia, UPM Serdang, 43400, Selangor, Malaysia.*
[2]*Department of Forest Management, Faculty of Forestry, University Putra Malaysia, UPM Serdang, 43400, Selangor, Malaysia.*

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ABSTRACT

A study was conducted on the effects of different incubation periods (1, 2, 4 and 6 days) on the level of microbial biomass carbon (MBC) in two soil Orders from open-land sites at Putrajaya (Prang Series, Order: Oxisols) and University Agriculture Park (TPU), Puchong, Selangor (Serdang Series, Order: Ultisols). A 100 m^2 plot was established at each site. About 10 soil samples were collected at the depth of 0-20 cm to form a composite sample. Five replicates were made out of the composite sample for each plot. The physical and chemical properties analyzed in this study include soil texture, bulk density, soil acidity (pH), electrical conductivity (EC) and soil organic carbon (SOC). The fresh soil samples were kept in chiller at 4 °C prior for analysis of microbial biomass carbon (MBC). Both soil series exhibited clay soil texture, where Serdang series had slightly higher amount of sand fraction and lower amount of clay compared to Prang series. Higher moisture and organic carbon also acted as a predisposing factor that causes higher growth of microbial biomass C in Serdang soil. The Serdang series soil samples can be subjected to dichromate digestion after 1st or 2nd day of incubation. In contrast, Prang series required a period of 2 to 4 days to achieve ideal amount of microbial biomass C prior for dichromate digestion. Further investigations on wider number of soil series needs to be carried out to obtain a better understanding on microbial biomass C incubation periods. The results obtained will be very beneficial in modifying the chloroform fumigation extraction and incubation timing as it can help in reducing the amount of time.

1. Introduction

Microbial biomass can be defined as part of microorganisms in the soil that vary in size between 5-10 μm^3, and decompose organic matters and crops residue such as fallen trees branches and leaves. Up to 5% of the total organic carbon and nitrogen in soil are contained in the microbial biomass, thus when the microorganisms dies, all of these nutrients will be released in forms that can be readily taken by plants [1, 2]. Hence, agricultural management influences soil microorganisms and soil microbial processes through changes in the quantity and quality of the plant residue entering the soil [3-5].

The Prang series, grouped under the order of Oxisols, possess the characteristic of very fine, oxidic isohyperthemic, developed from amphibolites and schist. The Prang series exhibits are red coloured, commonly being dark reddish to reddish brown throughout the soil profile. This particular series is also characterized by heavy clay texture and medium to fine sub-angular blocky soil structure. This series can be found on undulating areas. In contrast, Serdang series is a soil series under the order of Ultisols. Both soil series are categorized as highly weathered soils. However, Serdang series has a much lighter colour of yellowish red as compared to Prang series. The Serdang series is fine loamy and isohyperthermic, developed from sedimentary rocks.

Microbial biomass evaluation is found to be one of the most effective ways to evaluate the current quality of land fertility, especially in agricultural land [6]. Several methods are used to determine the level of the microbial biomass in the soil which includes fumigation-incubation, fumigation-extraction, and the substrate-induced respiration methods [7, 8]. Among all those methods mentioned, the most favourable method would be the chloroform fumigation-extraction method by [9-14]. The amount of soil samples needed to be incubated also varied based on the type of soil collected. Furthermore, many scientific studies on microbial biomass implements different incubation spatial where technique might not be suitable if large samples and many areas are to be evaluated at a short period of time.

Studies on the effects of varied microbial biomass incubation period on different soil series are still lacking, especially in Malaysia. Malaysia has more than 100 soil series across the nation, and it will give us a great view on the distribution of the microbial biomass in different soil series. Furthermore, suitable incubation period can be a good reference or predictive point to enhance future studies of the microbial biomass in soil. The objectives of the current research were to determine the effects of different and influences incubation period of 1st, 2nd, 4th and 6th day on the growth of microbial biomass carbon in Serdang and Prang series. The main reason only two series were studied in this research because we want to scale down the complexity by not putting many series into comparison.

2. Experimental Methods

2.1 Soil Sampling

Composite samples were collected from post- oil palm plantation land at Putrajaya (Prang) (2.905530N, 101.715736E) and fruit orchards at University Agricultural Park (TPU), Puchong, Selangor (2.984484N, 101.646057E) in June 2014. A 100 m^2 plot was established at both sites. About 10 soil samples were collected randomly in the plot and mixed to form a single composite sample. Composite sample for physico-chemical properties analyses was air-dried for 48 hours and sieved, while fresh composite sample was kept chilled at 4 °C prior for analyses of microbial biomass. Five replicates were made out of each composite sample from each site.

2.2 Soil Physico-Chemical Properties Analyses

Selected analyses for physico-chemical properties were done to observe the impact of certain properties on the distribution of microbial biomass C [15]. Gravimetric method for was used for soil moisture determination [16]. Universal pipette method was incorporated for the analysis of particle size. Bulk density was determined using core ring method. Soil pH was measured at a ratio of soil to water of 1:2.5 and was read using calibrated pH meter [2, 12, 17, 18]. The soil electrical

*Corresponding Author
Email Address: daljitkaramsingh@yahoo.com (D.S. Karam)

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conductivity (EC) was read using EC meter [19]. Wet dichromate digestion was used to determine the soil organic carbon was implemented in this study.

2.3 Chloroform Fumigation Extraction

Chloroform fumigation extraction was used to determine the level of microbial biomass as described by Witt et al. [20]. Two subsamples of 10 g of fresh soil were weighed in two different crucibles and a third subsample of 10 g of fresh soil was weighed into a 150 mL conical flask. Chloroform-fumigation was done by placing the soil samples in desiccators with ethanol-free chloroform for 24 hours in a dark condition. Fumigated samples were removed after evacuating the desiccators using a vacuum pump to free off the chloroform. Incubation is done by placing fumigated and inoculated soils in to a 250 mL jar with the bottom lined up with 10 mL of water and containing 10 mL of 1 M NaOH in a separate small glass vial. The jars were then air-tightened and incubated for 1, 2, 4 and 6 days. A second set of non-fumigated soil was incubated in the same way. Carbon evolution was determined after the incubation. Carbon dioxide evolution was estimated by measuring CO_2 respired from the soil over a period of 10 days. The CO_2 trapped in 1 M NaOH was analysed by back titration with 1 N HCl after addition of excess 3 N $BaCl_2$. The amount of CO_2 respired from fumigated and non-fumigated was used to calculate soil microbial biomass in the Eq. 1.

$$\text{Biomass C} = (Fc - UFc)/kc \quad (1)$$

Where, Fc = CO_2 flush from the fumigated sample; Ufc = CO_2 produced by the control; kc = Constant

2.4 Statistical Analyses

The independent student t-Test was used to determine the significant differences between physic-chemical and biological properties analysed between Prang and Serdang soil series. As for microbial biomass analyses, the independent t-Test were implemented to compare the level of microbial after 3, 5 and 7 days of incubation. Regression analysis was implemented to observe the pattern of microbial biomass increments after 1, 2, 4 and 6 days of incubation.

3. Results and Discussion

3.1 Physico-Chemical Properties of Serdang and Prang Series Soil

Table 1 summarized the selected physical and chemical properties analysed to support the findings for the factors that might be the predisposing factors for varied microbial biomass C level at different incubation periods. Both soil series exhibited clay texture due to high content of clay fraction in each soil in which Prang showed a significant higher amount of clay (71.00%) compared to Serdang (48.50%) series soil. Moisture level at Serdang series soil was found to be comparatively higher ($p \leq 0.05$) than Prang series. This condition could be due to soil collected at Prang series soil was of an open area without any ground cover, while Serdang series soil was collected at an agricultural land soil. Electrical conductivity in Serdang series soil was also found to be significantly higher compared to Prang series. This result might be influenced by the application of fertilizer and drainage system management at area where soil was collected. Organic carbon was also found to be higher at Serdang as compared to Prang Series soil. Both soil series showed an acidic soil with pH less than 6.0 whereby proved that the soil has experienced or undergone high weathering activities on its soil.

Table 1 Physico-chemical properties of Serdang and Prang series

Parameter	Serdang	Prang
Clay (%)	48.50 ± 1.27a	71 ± 1.27b
Silt (%)	14.00 ± 7.85a	12.80 ± 1.58a
Sand (%)	37.90 ± 8.07a	15 ± 1.27b
Bulk density (gcm^{-3})	1.15 ± 0.03a	1.38 ± 0.03a
Moisture content (%)	3.74 ± 0.08a	2.23 ± 0.10b
Acidity (pH)	5.16 ± 0.33a	5.09 ± 0.03a
Electrical conductivity (dsm^{-1})	1.48 ± 0.57a	3.49 ± 0.51b
Organic carbon (%)	1.34 ± 0.06a	0.69 ± 0.11b

Note: EC, electrical conductivity; Different letter indicated significant differences at $p \leq 0.05$ *using* t-*Test.*

3.2 Soil Microbial Biomass Carbon in Two Soil Series with Different Incubation Period

The result from analysis of variance showed that there were significant differences between the soil series for the organic carbon determination. Significant differences were noticed between Serdang and Prang soil series. Serdang soil series showed a higher amount of microbial biomass C compared to Prang on the 1st, 2nd, 4th and 6th day respectively (Table 2). The linear regression analysis also showed that the deviation of the slop had a highly significant difference (Table 3 and Figs. 1-2).

Table 2 Microbial biomass C level in Serdang and Prang soil series at different incubation period

Day interval	Serdang	Prang
1st day	477.28 ± 6.54a	330.88 ± 3.41b
2nd day	427.74 ± 2.77a	265.12 ± 1.48b
4th day	367.30 ± 12.15a	245.60 ± 6.91b
6th day	271.50 ± 3.65a	193.12 ± 2.43b

Note: Different letters between soil series indicate significant differences at $p \leq 0.05$ *using* t-T*est.*

Table 3 Comparison of microbial biomass C level increment for Serdang and Prang soil series

Day interval	Serdang	Prang
1st day	477.28 ± 6.54a	330.88 ± 3.41a
2nd day	427.74 ± 2.77abc	265.12 ± 1.48bc
4th day	367.30 ± 12.15c	245.60 ± 6.91c
6th day	271.50 ± 3.65d	193.12 ± 2.43d

Note: Different letters within the same column for each soil series indicate significant differences at $p \leq 0.05$ *using ANOVA and Tukey's post hoc test.*

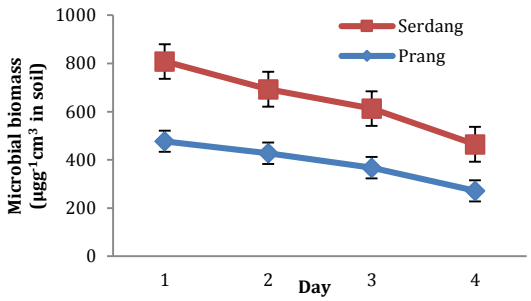

Fig. 1 Microbial biomass C level in Serdang and Prang distribution at 1st, 2nd, 4th and 6th day incubation

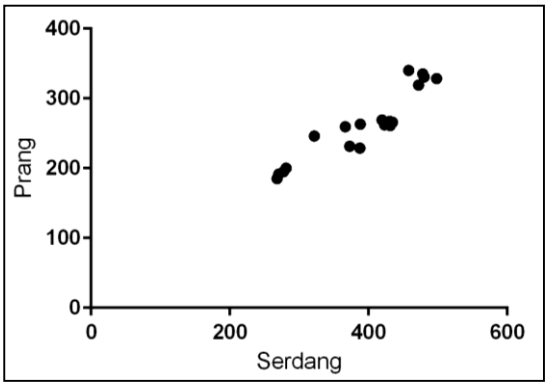

Fig. 2 Linear regression analysis graph of the distribution of microbial biomass C in Serdang and Prang soil series

In Table 2, Serdang series, the 1st and 2nd day of incubation showed no significant differences. Despite the slightly lower level of microbial biomass C in the 2nd day incubation period compared to 1st day for Serdang series, the differences can be neglected. In contrast, the 2nd and 4th day incubation period of Prang series yield no comparative differences. Hence, soil analysed for microbial biomass C determination for Prang series can be done right after the 2nd day of incubation.

The high content of organic material such as organic carbon in soil facilitates the soil microbial activities and thus, contributes towards a higher number of microbial biomass C in the Serdang series. In contrast, the level of microbial biomass carbon decreases as the period of incubation increases for both soil series. Soil with high pH, salinity and fertility all have been connected with increasing importance of bacterial function in soil. Electrical conductivity (EC) did show a significant

difference for both soil series. The highest values recorded are for Prang soils. This shows that Prang series have higher salinity compared to the Serdang series. Soil microorganism activity declines as soil EC increase [21, 22]. Lower bulk density value possessed by Serdang series soil also act as an essential factor that gave a significant rise to microbial biomass C levels in the Serdang series.

Based on the analyses carried out, times span needed for a successful and ideal incubation of soil with chloroform was found to be either on the 1st or 2nd day after incubation for Serdang soil. This means that immediate analyses of dichromate digestion can be done. In contrast, Prang series took a longer incubation period to be suitable for dichromate digestion. Immediate analyses can be done right after 2nd or after the 4th day of incubation. The reason is because of the nature of the Prang series used in this experiment that contains low amount of microbial biomass that could be affected by the soil compaction, moisture and surrounding environment.

4. Conclusion

Chloroform fumigation extraction is widely used for the determination of microbial biomass in soil. Many publications use different time intervals or period of incubation. The factors considered include type, size and history of the land usage of that particular soil. In this study, the two soil series, Serdang and Prang series, were subjected to different incubation periods of 1, 2, 4 and 6 days. Selected physical and chemical properties also had been analysed. Both soil series exhibited clay texture. Serdang series possess higher amount of sand fraction compared to Prang series. This characteristic gave Serdang series a lower soil compaction and higher moisture retention in soil which provides a suitable environment of the microbial biomass growing as compared to Prang series. Ideal incubation period for Serdang series was found to be either 1 or 2 days, while Prang series soil needed to be left incubated for 2 days or up to 4 days. It is recommended that further and continuous studies to be conducted on the effect of varied incubation period on a wider range of soil type, as it helps in improving the technique used in the determination of soil microbial biomass especially on saving time and we would be able to analyse many samples in a short period of time.

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