Does different management and organic inputs in agroforestry system impact the changes on soil respiration and microbial biomass carbon?

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Abstract. Agroforestry system is assumed to be an alternative system which reduces the negative impact of the forest conversion to agricultural land in term of maintaining soil quality and its fertility. This study was aimed to explore effects of different management on agroforestry system upon soil microbial biomass, soil respiration, and organic matter dynamic and nutrients. This study was conducted at UB Forest from November 2017 to February 2018 by collecting various parameters and soil samples from eight different types of land use. Biomass were measured using allometric equation by collecting axis diameter at the height of 1.3 m. Litter inputs were collected using a litter trap method, while in-situ litter were determined using destructive methods. Soil carbon was examined using Walkley and Black method and POM C. Biplot and CVA multivariate analyses were adopted to determine the impact of different management and organic matter inputs. The result showed that the conversion from old agroforestry system of Pine Coffee (PK3 and PK4) to agriculture practices PS (Pine 40 years and vegetable (cabbage) reduced the organic matter inputs by 40%, litter input by 80%, and total POM C by 40% The highest soil microbial biomass carbon and soil respiration were detected on PK3 plot (pine 35 years with coffee at 5 to 8 years old) which were to about 55.48 mg kg⁻¹ and 4.03 mg, respectively. The reduction on organic matter inputs highly correlated to the level of soil respiration and microbial biomass C. Multivariate analysis can distinguish and clustered the treatments along X and Y axis to about 90%. The best system to provide the greatest input of soil organic input which then affected the highest soil organic carbon and microorganism. However, the consequence of this was the increasing of soil respiration and releasing carbon to the atmosphere.

1. Introduction
Soil microbial biomass carbon (SMB-C) and soil respiration are closely related to several functions of microorganisms, such as ammonifiers and nitrifiers (Andrade et al., 1995), populations of legume-nodulating bacterial [1] and enzyme activity in the soil [2]. Furthermore, SMB-C is a determinant of the diversity and sustainability of biochemical reaction processes in the soil. Theses were including the process of decomposition of soil organic matter (SOM), nutrient cycles, dissolving inorganic phosphates, degradation of xenobiotic components and pollutants, soil structure development,
improving deposits of SOM or carbon sequestration [3]. Biological control and suppression of pathogens that harm plants and reducing the outbreaks and for all functions above SMB-C is often used as an important component for maintaining soil quality and increasing land and plant productivity [4, 5]. Several studies have shown that, activity, biomass and microbial abundance depend on existing vegetation so that litter diversity stimulates microbial diversity [6]. He also stated the results of his research that the diversity of organisms in the forest is directly proportional to the abundance of microbes, which causes the biological soil to become more active.

Previous studies that looked at the effects of land use changes that are closely related to reduction of the input of organic matter (OM) in which it was in accordance to the decreasing of SMB-C in the soil [7]. In the Amazon region of Brazil where SMB-C in the forest area has a value of 254-796 mg C kg\(^{-1}\) of soil, the value will decrease to 166-567 mg C kg\(^{-1}\) of soil when it was converted to seasonal crops, and when it becomes pasture the value ranges from 93-623 mg C kg\(^{-1}\) of soil [8, 9]. The microbial abundance conditions and some types of decomposers can vary with similar litter decomposition. But in various types of litter, which are commonly found in forest ecosystems, microbial populations tend to be stable and changes occur insignificantly. In the context of this study, the important thing in measuring SMB-C and its response is in order to evaluate the extent to which SMB-C can be used as a sensistive indicator of changes that occur in an agroecosystem [10, 11].

According to Mande [12], the conversion of forest land into cultivated agricultural land can result in a decrease in the amount of organic matter and nutrients produced, as well as causing an imbalance in soil hydrological function that will result in increased carbon dioxide (CO\(_2\)) emissions from the soil. The amount of CO\(_2\) from the soil is influenced by the activity of soil microorganisms in producing CO\(_2\) which is determined by the input of organic matter as a source of soil C reserves [3]. According to Hanafiah [13], organic matter is a source of energy and C for microorganisms in producing CO\(_2\) very closely related to land management carried out by farmers. Information on the influence of land management on the process of soil respiration is also very limited, especially in the agroforestry system.

The UB Forest area is a limited protection forest area where intensive agricultural activities are still very limited [7]. Some part of UB forest's land area has been converted into agricultural areas, cultivation and residential areas. The area which is an educated forest with an area of about 540 ha which is mainly a production forest stand dominated by timber species of mahogany (Swietenia mahogany) and pine (Pinus merkusii) with various types of flora that are under stands such as taro (Coelacasia esculenta), carrot (Daucus carota), coffee (Coffea sp), ginger (Zingiber sp) etc under agroforestry system. Agroforestry system is assumed to be an alternative system which reduces the negative impact of the conversion in term of maintaining soil quality and its fertility [14]. These various conditions of land management were hypothesized to affect the diversity and activity of soil microbe particularly fungus and bacteria. Important information about SMB-C baseline in this area is very important in assessing the condition of soil quality with the indicator value of SMB-C along with the measurement of soil respiration as an indicator of soil microbe activity. With a high SMB-C value it shows that the amount and activity of soil microbes is still good, which in turn will have an impact on the sustainability of nutrient availability in the soil and the sustainability of the ecosystem. The absence of preliminary data or baseline will make it difficult for management to evaluate whether the land use of the land carried out in the future is better than the beginning or not whether there has been a degradation in the quality of the land.

This study was aimed to analyze the impact of different inputs of organic matter (OM) from several land uses due to changes from forest conditions to other land uses such as: pine monoculture, pine + coffee with different age stands, and compared to mahogany + taro land, mahogany + coffee and vegetable farms, towards SMB-C, soil respiration, and its relationship with soil organic matter fraction and total microbial population. The schematic of the background was presented in Figure 1.
2. Methodology

2.1. Site Location
The study was conducted in Sumbersari, Sumberwangi and Buntoro, Tawangargo Village, Karangploso-Malang-East Java-Indonesia. The geographical coordinates of this region are located at 122°35'06.62'''-122°37'53.19'' E and 7°55'14.54'''-7°52'27.24'' S. Relief of this region was hilly which is located at an altitude between 700-1250 meters above sea level (Figure 2). The distance from the center of Malang is about 20 km to the west, which can be reached by land travel which takes about 30 minutes. The history of land use in the past was a production area of pine and mahogany forests under the authority of Perhutani (Forest Agency Unit) which was then transferred to Brawijaya University in 2016 which covered an area of 514 hectares and nowadays were known as UB Forest. At present there are approximately 800 farmers who cultivate land in this area with an agroforestry system that has major commodities of coffee. Besides coffee, there are also other crops that are cultivated such as taro, chili, cabbage, carrots, etc. But unfortunately, management in this area is very diverse and causes low levels of crop productivity.

UB Forest is one example of a forest ecosystem in Malang Regency. Since 1976, most of the use of forest land has been converted into agroforestry system with primary crops in the form of pine and mahogany covering an area of 392.58 ha, while some plots have become agricultural land covering 81.42 ha for local residents and 50 ha in the form of protected forests. Changes of the forest function cause a change in the composition of plants in UB Forest's land which directly affects the ability of plants to store carbon stocks and produce litter as a microbial food source and material for soil organic material deposit.
2.2. Experimental design

The research design used in this study was a Randomized Block Design (RBD) with eight observation plots with three replications, so that the number of observation plots was obtained as many as 24 plots. Selected observation plots and their characteristics of land are presented in Table 1.

| Code | Plot characteristic                          |
|------|---------------------------------------------|
| PK1  | Pines (20 years) + Coffee (4-5 years)        |
| PK2  | Pines (30 years) + Coffee (4-5 years)        |
| PK3  | Pines (35 years) + Coffee (4-5 years)        |
| PK4  | Pines (40 years) + Coffee (4-5 years)        |
| PM   | Pines (25 years)                             |
| PS   | Pines (40 years) + Vegetable (Cabbage/Carrot)|
| MT   | Mahogany (40 years) + Taro                   |
| MK   | Mahogany (40 years) + Coffee (4-5 years)     |

Variable to be examine were consisted from vegetation and soil aspect. The variable of vegetation aspect to determine were involved non and destructive sampling.

| Aspect     | Variable                                      | Method                                         |
|------------|-----------------------------------------------|-----------------------------------------------|
| Vegetation | Tree diameter (cm) for estimating biomass and basal area (BA) | Non destructive (measuring tape)               |
|            | Litter insitu (g m²)                          | Destructive (metal frame 50 cm x 50 cm)        |
|            | Litter input (g m²)                           | Destructive (litter trap 1 m x 3 m)            |
|            | Understory biomass                            | Destructive (metal frame 50 cm x 50 cm)        |
Soil pH (H$_2$O)
Soil organic matter (%)
Soil water content (%)
Soil bulk density (g cm$^{-3}$)
Soil respiration (kg C-CO$_2$ ha$^{-1}$ day$^{-1}$)
Soil microbial biomass
Soil microbe (cfu ml$^{-1}$)
Soil organic matter fraction

| Soil      | Soil pH (H$_2$O) | Glass electrode pH meter |
|-----------|------------------|--------------------------|
|           |                  | Walkley & Black          |
|           |                  | Gravimetry               |
|           |                  | Ring cylinder            |
|           |                  | KOH trap                 |
|           |                  | Fumigation               |
|           |                  | Total Plate Count        |
|           |                  | POM C                    |

3. Results and discussion

3.1. Plot characteristic

Based on Table 3, it showed that there was a significant difference (p < 0.05) to the value of Basal Area (BA) between treatments. BA reflects the stand density calculated based on the number of trees and the diameter of trees in a land which will affect the amount of plant biomass and litter input. The amount of BA at plot PK4 showed the high density of trees that have the highest BA value at about 39.04 cm$^2$ m$^{-2}$ and the lowest BA value was found at MK production forest with a value at about 24.38 cm$^2$ m$^{-2}$.

The age of tree can affect the average size of tree biomass based on their diameter. The measurement results showed that the highest biomass average was found in the use of MT at the age of 40 years to about 424.5 tonne ha$^{-1}$ while the lowest average tree biomass was found at PK1 with the age of 20 years which was had a total tree biomass of 201.7 tonne ha$^{-1}$. This was proved that tree biomass is influenced by the type and age of tree, thus producing different biomass values [15]. According to Uthbah et al. [16] the amount of biomass will increase in line with increasing stand age so that the input of organic matter will also increase in line with increasing of tree age.

### Table 3. Basal area (BA), Tree biomass dan Understory across different land use

| code | Basal Area (BA) cm$^2$ m$^{-2}$ | Tree biomass ton ha$^{-1}$ | Understory g m$^{-2}$ |
|------|--------------------------------|---------------------------|----------------------|
| PK1  | 29.19                          | 201.7 a                   | 17.02 bc             |
| PK2  | 37.73                          | 260.7 ab                  | 10.09 ab             |
| PK3  | 29.13                          | 255.7 ab                  | 25.27 c              |
| PK4  | 39.04                          | 417.0 cd                  | 23.43 bc             |
| PM   | 29.96                          | 218.1 ab                  | 23.45 bc             |
| PS   | 26.09                          | 260.7 ab                  | 2.76 a               |
| MK   | 24.38                          | 319.7 bc                  | 17.84 bc             |
| MT   | 32.66                          | 424.5 d                   | 21.66 bc             |

Besides tree biomass which is stored in trees stem, biomass is also deposited in the understorey. Understorey that grown on the soil surface will be able to retain organic matter as native vegetation adapted to shading condition underneath the main tree [17]. The content of C and plant biomass is influenced by the type of the crop but mainly it contribute to about of 50% of the total biomass [18]. The results of observations of understorey measurements obtained the highest number of understorey was found at PK3 with a value of 27.69 g m$^{-2}$ and the lowest was detected at PS with a value to about 2.76 g m$^{-2}$, approximately only 10 % to those value of PK3. Biomass is also stored in litter form. Litter is one component in the forest that can also store C and sources of organic material that is above
the ground or forest floor [19]. Plant biomass is a major component that increases the accumulation of C-organic nutrients, root growth and microbial activity [12].

3.2. Soil organic matter input

3.2.1. Litter dynamic

Litter input measurement and collection were carried out every 2 weeks to monitor the dynamics of litter inputs produced by tree, using a 1 m x 3 m litter trap. The effect of different land uses on the average litter dynamic measured from total accumulation of litter dry weight showed a significant effect (p<0.05). MK plots had the highest litter accumulation amongst other plot during the period ok week 4 to week 8, while PK3 and PK4 showing consistent litter input during the period of observation at about 0.33 t ha⁻¹. PS land use system provides the lowest litter input at about 0.03 t ha⁻¹ which was only 10% lower than of those the litter input of PK3 or PK4 plots (Figure 3).

![Litter input over the period of observation across land use system](image)

**Figure 3.** Litter input over the period of observation across land use system

3.2.2 Litter in situ

Litter in situ measurement and collection were carried out by destructive sampling to monitor the total of litter which is deposited at soil surface over the period of cultivation, using 0.5 m x 0.5 m metal frame. From the figure 4, it can be seen that the effect of land use to the average dry weight of litter in situ were found to be significant (p<0.05). The dry weight of litter in situ on PS (pine trees aged 40 years with annual crops) had the lowest accumulation of litter to about 23.04 t ha⁻¹ due to minimum litter inputs to this system, while the highest average yield of in situ litter dry weight was found at PK3 plots which was valued at 130.28 t ha⁻¹. In situ litter dry weight of PK3 higher to about 82.3% compared to PS plots.
3.3 Soil chemical properties

3.3.1 Soil C-organic matter

C-organic content depends on the balance of litter input by plants and the process of litter decomposition by soil microorganisms [20]. The results showed that the level of C-organic soil on PS, i.e. forest land that has been converted into simple agroforestry land (pine and annual crops such as cabbage) is relatively low which was significantly different (p<0.05) to the plot of PK3, PK4, PM, and MT. From all observations, the highest C-organic content was found in PK3 land which was valued at 5.8% and the lowest C-organic content was found in PS plot which was valued at 3.02% (Figure 5). Significantly decreased levels of C-organic from PK3 land to PS as much as 2.78%. The level of C-organic on PK3 plot has the highest value compare to than other plot and is significantly different (p<0.05) from the value of C-organic on PS and PK1 plot but not significantly different (p<0.05) from PK2, PK4, PM, MT and MK plot. Previous studies have proven that C-organic levels decrease when conversion of forest land to agricultural land [20]. Changes in land use can accelerate decrease of soil C-organic [21]. If litter input is limited, the source of organic material available on soil also decreases, thereby it will be affecting levels of C-organic and microbial activity.
Land use systems affect the availability of soil organic matter Chen et al. [21]. PS plot in the form of pine and annual crops which mostly dominated by vegetables (cabbage or carrot), decomposition process is accelerated by intensive land management. Pruning activities towards pine tree branches affected to reduce input of litter, while litter input from understorey is also limited. Furthermore, all yields from vegetables was not returned back to the field, resulted in low soil C-organic. At PK3 and PK4 plots which has high levels of C-organic was due to all organic inputs were to remain on the surface of the soil (leaves, branches, understorey, etc), no tillage is carried out, so that decomposition runs naturally by soil microorganisms (fungus and bacteria) by releasing various organic acid and enzymes.

The labile soil C organic at PK3 and PK4 were higher than the other land use, which determined by POMC method. This fraction was becoming the source of energy for soil microorganisms including soil bacteria, with ratio at 0.54 to 1.09.

3.3.2 Soil pH
From the analysis of the variance, the effect of differences in land use on soil pH showed a significant difference (P <0.01). In detail, soil pH values in PS land were not significantly different (p<0.05) to soil pH at PM and PK2 plot, but significantly different to soil pH under PK3 plot (Figure 6). Generally, it was found that the lowest soil pH value was found in PS plot which was to about 4.5, while the highest soil pH value was found in PK3 plot, 5.8. Significantly decreasing on soil pH from PK3 to PS as much as 22.41% were observed. Soil pH on PK3 plot was the highest and significantly different from the soil pH value on PS, PM, PK1, PK2, PK4, MK4, and MT plot. Soil pH value on PK3 land which is 5.8 is classified as slightly acidic, while on PS land which is 4.5 classified as acidic.

The soil pH value is related to the abundance of soil microorganisms, especially bacteria. The results of the observations showed that the pH of the soil in the observed land ranged from 4.5 - 5.8. The soil pH value at the study site is in accordance with the bacterial living conditions, supported by the statement of Cahayaningtyas and Sumantri [22] that the optimum pH for most bacteria is a minimum of 4 and a maximum of 9.

3.4 Soil biological properties
3.4.1 Microbial biomass soil
The effect of different land uses on soil microbial biomass C were found to be significant (p<0.05). Microbial C biomass at PK3 and PK4 plot was not significantly different, but significantly different to microbial C biomass at PS plot. From all observations carried out, it was found that the highest soil microbial C biomass was found in PK3 land which was valued at 55.48 mg / kg of land while the lowest soil microbial biomass was found at PS plot was to about 43.93 mg / kg of soil (Figure 6). Changes on land management from PK3 to PS was reduce soil microbial biomass C by 20.81%. In addition, PK3 soil microbial biomass C plot even though it was different to PS plot, but it was not significantly different to PK4 and MT plot.

SMB-C is an indicator that is susceptible to the dynamics of organic matter because the microbial fraction changes in a short time and the differences are easily detected [9]. The microbiology of soils such as microbial biomass is used as an indicator that is susceptible to soil quality, with a comparison of different land uses. The high microbial activity is used as a basis for improving soil quality. Soil microbial biomass is a potential source of nutrients for plants, higher microbial biomass shows a high level of soil fertility [23]. Increasing the number of bacterial populations in microbial activity is caused by the accumulation of organic matter which is positively correlated with microbial abundance and microbial respiration.
Increased microbial biomass results from an increase in soil organic C [24]. Microbial activity is also influenced by litter quality [6], in conditions of low litter quality, microbial populations will be at a low level (stress level) resulting in decreased mineralization and decomposition of C-organic and increasing carbon release to atmosphere. Addition of litter has a significant influence on the microbial population [25], increasing the number of soil microbes is supported by the availability of nutrients and the usefulness of these microbes [26]. Litter originating from different canopies will produce high biomass and produce high biological activity inside and at the surface of the soil [27].

3.5 Total Soil Bacteria Population
Land use affected significantly (p<0.05) to the number of soil bacterial populations whereas the total bacterial population on PK3 land was significantly different from other plot (PM, PK1, PK2, PK4, MK and MT) and it was reached the highest population (Figure 7).

Total bacterial populations at PK3 plot was valued at $10.1 \times 10^6$ cfu ml$^{-1}$ while the lowest total bacterial population has been detected at PS plot was to about $1.4 \times 10^6$ cfu ml$^{-1}$ which was only 10% compare to the abundance of soil bacteria at PK 3 plot. There was a significant decrease in the total bacterial population by 86.3% (Figure 8). The high level of soil bacterial population is related to the availability of litter as an abundant source of soil organic material therefore the total population is also become high. Conversion of forest to agricultural land causes a decrease in soil fertility from both chemical and biological indicators [28]. Microbes and C elements are generally more sensitive to changes in land use than physical properties [29]. For example, microbial biomass decreases faster after changes in land use [30]. Soil microbes are one of the healthy bioindicators of the soil, because they play a role in the process of decomposition of organic matter into nutrients. Soil, soil and plant microbes play an important role in the biogeochemical cycle and recycle elements C, N, P and other minerals [31].
**Figure 7.** Total soil bacteria across different land use system

**Figure 8.** Colony of soil bacteria at PK1, PK2, PK3 and PK4 plot
Soil microbial activity is supported by several factors including the availability of food sources, habitat suitability, and interactions with other organisms. Soil organic matter and soil microbial C biomass are found to be decreasing in natural forests that have been converted into agricultural land [32]. The high biodiversity of microbes is present in soils with different vegetation. Solihin and Fitriatin [33] added that, forest ecosystems are a habitat that has high biodiversity that supports high microbial growth. Guillaume et al. [30] stated that, the biological nature of land, especially microbial biomass is a factor that is more sensitive to changes in management and land use.

3.6 Soil respiration
Measurement of soil respiration uses a closed system called the titrimetry method that determines CO2 in closed plastic jar trapped by KOH base solution has been successfully recorded soil respiration rate in which it was significantly different (p<0.05) across the treatment (Figure 9). The lowest soil respiration value has been detected at PS plot and PK1 plot, while soil respiration at PK2, PK3, PK4, PM, MK, and MT plot was found to be similar. Soil respiration is believed to be influenced by changes in land management that impacted on the availability of organic material sources. That is because the remaining organic material not only functions as a source of nutrients, but also can increase the number of microorganisms and microorganism activities in the soil [34]. Soil respiration can be used to evaluate the ability of biodegration C and evaluate the status of organic matter [35]. The C-organic content in litter input determines the quantity of soil C deposits.

The analyses of correlation showed that litter in situ had a positive and strong relationship to soil C-organic and soil microbial biomass, with $R^2 = 0.47$ and 0.49 respectively. The accumulated litter then enters the decomposition stage through the actions of soil microorganisms, resulted to the increasing of soil C-organic content and microbial C biomass (Figure 10). It also can be seen that microbial C biomass had a strong and positive relationship with soil C-organic content and total soil bacterial population, resulted in the $R^2$ values at about $= 0.55$ and 0.37 respectively. Soil C-organic content has a positive and strong relationship with the total bacterial population obtained $R^2$ value was
to about 0.38 while the relationship between soil microbial biomass C was also has a positive relationship with the total bacterial population produce $R^2$ was to about 0.24 (Figure 10).

The results of this study are in line with the statement of Fang et al. [20]; Moscatelli et al. [36], whereas a positive correlation between microbial C biomass and soil C-organic content is caused by microorganisms that are heterotrophic and have biological activities that depend on organic matter. The increase in microbial C biomass is caused by increased C-organic and litter accumulation [24]. Conversion of forest land into monoculture system occurring on PS plot can affect soil C-organic content and have an impact on the decrease in microbial C biomass. In addition, litter in situ and C-organic content are the lowest compared to other land uses, this is due to pruning of pine branches and tillage so that it affects the decrease of C microbial biomass in the land. Yang et al. [37], argues that on land without tillage and minimal tillage can increase the growth of mycorrhizae, fungi and can significantly increase microbial biomass that plays a role in decomposing carbon accumulation. At PK3 plots which is mainly becoming agroforestry system without any tillage practices, with vegetation in the form of pine, coffee and various understorey has the highest microbial C biomass value and is significantly different from other land uses that is 55.48 mg kg$^{-1}$. Yadav [23] explains that soil microbial biomass is an indicator of soil fertility, where microbial biomass is part of organic material consisting of living things in the form of microorganisms.
Using multivariate analysis (Biplot), the effect of various variable was examined. It can be seen from Figure 10 that the magnitude and direction of each variable can be plotted, whereas litter had a close relationship to soil pH and bacteria. Those relationship was also can be identified using an angle of those variable which <45 degree. Meanwhile understoery had similar magnitude and direction with C-storage and soil respiration. All those variables contribute to the total significantcy to about 99.41% with PC 1 and PC2 at the value of 96,76% and 2,65 %, respectively (Figure 11). This analysis has been use in previous study to determine the impact of land management on soil macrofauna [7].
4. Conclusion

Soil microbial C and litter are part of the organic material. If litter as an abundant source of food is available in soil, it can support microbial activity so that the bacterial population in the land also increases. Total bacterial populations are found in soils that have suitable properties and allow soil microorganisms to be active and reproduce. Appropriate soil conditions include soil pH, sufficient water and energy sources in the form of sufficient organic matter, types of plants related to litter quality, and land use practices related to tillage. In land use where there is no tillage, it has natural conditions that can support soil fertility, for example, there are tree plants as the main supplier of litter input in the land, understorey and litter that cover the soil surface, with litter can be a major food source of microorganisms, understorey can condition the climate micro (temperature and soil moisture) so that in accordance with the conditions of growing microorganisms and protect the soil surface from direct sunlight and rain water so that it can maintain soil organic matter and soil microorganism activity as an indicator of soil fertility. The soil quality under PK3 plot are the most promising condition to maintain soil microorganism activity and sustainability of the soil environment.

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