# A study on the practical use of compost made from pruning materials based on mineralization characteristics

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# A study on the practical use of compost made from pruning materials based on mineralization characteristics

（分解特性に基づく剪定枝由来堆肥の活用に関する研究）

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# General abstract

As we know, composting of pruning materials and applying into soils is a sustainable approach to dispose the pruning materials and improve soils. However, the practical use of pruning materials compost (PM) is undetermined. Therefore, this study was to propose how to use the compost made from pruning materials to improve soil fertility and promote the plant growth. This study consisted of (1) C mineralization characteristics of PM, (2) Effect of PM application on plant growth and (3) Effect of PM application on soil organic matter content and biological properties.

The results indicated that comparing with conventional compost, application of pruning materials compost (PM) into soil, supply more mineralizable C as resource to microorganisms thereby maintain microbial activity in soil for longer time. And the activation energy for PM mineralization is almost same with other conventional plant materials compost and is larger than dung compost.

Application of sufficient PM into soils with different organic matter content and microbial activity, increased soil total C, N content and microbial activity obviously, indicated that application of PM into soil increased soil organic matter content obviously because of the large amount of organic matter content in PM, meanwhile contributed to improve soil biological properties. Especially in soils with low microbial activity, application of PM into soils increased the soil organic matter and microbial activity last for longer time.

Through comparing with effect of PM on plant growth of Leguminous plant and

Brassicaceae plant, because of the relative larger amount of mineralizable C content in PM, in soil with low microbial activity, application of PM inhibited Brassicaceae plant growth in early stage, however there is no inhibition effect on Leguminous plant growth which can fix N itself. In soil with high microbial activity, application of PM into soil, relative faster mineralization contributed to faster turnover of PM in soil, therefore supply available nutrients to plants thereby promoted plant growth immediately. Therefore, through comparing the plant growth of Leguminous plant and Brassicaceae plant, application of PM lead to N starvation thus inhibit plant growth in soil with low microbial activity. And more PM application caused N starvation more obviously. Meanwhile in addition to N starvation, there is no negative effect on plant growth.

Therefore, PM can be widely applied into different soils for improving soil properties and accelerate plant growth with appropriate application method. Because of the large amount of mineralizable C, PM application maintain soil microbial activity for long time, thereby improve the soil biological properties thus improve the soil capacity of green recycle. In soil with high microbial activity of agriculture or in green space with deep root vegetations such as forest, grassland and so on, application of PM improve soil properties meanwhile promote plant growth. however, in soil with low microbial activity, longer process of PM mineralization lead to N starvation limit plant growth. Therefore, amend soil with low microbial activity of agriculture or in green space with shallow root vegetations, three methods can be selected. (1) Controlling application amount of PM at first with a small amount PM application, however because of the small application amount, the exist period is short, therefore the PM need to be applied more often, and with soil microbial activity increasing, increasing the application amount and decrease the application frequency. (2) Large amount of PM to improve soil organic matter content and biological properties in soil with low microbial activity likely lead to N starvation in soil, N fertilizers or high N content materials can be used together with PM to amend soil with low microbial activity. (3) In addition to applying N fertilizers or high N content materials together, setting maturity period for PM in soil is another method to avoiding inhibition on plant growth in soil with low microbial activity.

# Chapter 1 General introduction

## 1. 1 Production of pruning materials

In urban areas, the quantity of plant waste, such as pruning materials, has been consistently increasing with the rapid development of urban green spaces (Zhang et al. 2013). Pruning materials constitutes approximately 45% of the annual wood growth of some species of trees in Japan (Fujiwara et al. 2003), and the planting of trees in metropolitan areas has become increasingly popular in industrialized countries. In 2011, in Tokyo, Japan, the Basic Policies for the 10-Year Project for Green Tokyo were issued (Tokyo Metropolitan Government 2007). Until April 2017, the numbers of border trees in Tokyo have reached 944166, had almost achieved the original goal of 10-Year Project for Green Tokyo, until April 2018 the area of park had reached 7839ha, have surpassed the goal of 10-Year Project for Green Tokyo. Notably, this gradual increase of border trees has considerably increased the amount of pruning materials. Furthermore, the amount of pruning materials produced in parks is already substantial. For example, in Tokyo, the output of pruning materials in Yoyogi Park, Akatsuka Park, and Hikarigaoka Park are 3.7, 3.1, and 5.1 $m^3$/ha/year, respectively (Tsukuda et al. 2009). Thus, in a manner of speaking pruning materials constitute a large proportion of landscape waste (Zhang et al. 2013).

However, in past the mainly approach of disposing pruning materials is burning, burning of pruning materials had caused environment load such as air pollution and greenhouse gas production. Consequently, the disposal of pruning materials has become a major problem that affects the environment and inhibits sustainable development (Tuomela et al. 2000).

## 1.2 Green recycle of pruning materials

In urban green space, mulching wood chips made from pruning materials on soil surface and composting of pruning materials then applied into soil are considered as green recycle in recently (Kanbara et al. 2016). Mulching of pruning materials on soil can increase the soil C obviously, meanwhile mulching of pruning materials on soil surface prevent weed obviously (Takahashi et al. 2008). However, mulching of pruning materials inhibit the nursery trees growth as well (Takahashi et al. 2001).

Replacing the traditional method of burning pruning materials with sustainable approaches for its recycling, mulching of pruning materials on soil is a sustainable approach to disposal pruning materials and improving soil properties (Takahashi et al. 2008), meanwhile Takahashi et al. (2000) found that compared with forest soil, soil in urban parks often lacks organic matter. Tsukuda et al. (2009) reported that application of pruning materials on the ground in urban parks increases the organic matter content, carbon (C) mineralization rate (microbial activities), and available nutrient contents. In addition, the microbial activity in soil is the insure for green recycle. Therefore, how to maintain the soil microbial activity is also necessary to make sure the process of green recycle.

However, application of immature or unstable fresh plant materials to soils may bring about problems such as Nitrogen (N) immobilization by microorganisms, anaerobic conditions, accumulation of phytotoxic substances (for instance acetic acid, phenolic compounds or ammonia) that could inhibit seed germination and root growth

(He et al. 2000). So that recycle pruning materials with mulching on soil directly maybe limit plant growth thereby limit planting or maintaining plant growth in green space. Composting is also a part of green recycle. Mulching of pruning materials although can be used to dispose pruning materials sustainable as a part of green recycle, however the negative effect on plant growth limit the usable range. While composting of organic materials into compost can reduce the mineralizable C content and reduce the unstable factor contributed to limiting plant growth.

Therefore, composting of pruning materials and mixed them into soils is a better sustainable approach to disposal of pruning materials and improve soil properties instead of mixing pruning materials into soil directly to avoid the negative effect on plant growth.

## 1.3 Composting of pruning materials

Composting is one of the best choices for green waste treatment because it is an environmentally friendly process, adds value to a wide variety of organic waste, and the compost obtained is suitable as an amendment for soils and thus enables waste to be recycled (Guardia et al., 2010; Vargas-García et al., 2010). Organic matter is transformed during composting as a result of complex interactions among chemical, physical and biological processes (Tejada et al., 2009). Comparing with mulching with pruning materials into soil directly, composting of pruning materials and mixing it into the soil also be effective for the maintenance of soil organic matter content and microbial activity and nutrient cycling (Melero et al. 2007), can enable the optimum use of pruning materials, both economically and substantially, and effectively decrease $CO_2$ emissions (Boldrin et al. 2009). In addition, composting of pruning materials reduce the mineralizable C, also reduce of the phenolic substance content, thus improves soil properties meanwhile decrease the negative effect on plant growth and seed germination (Benito et al. 2003). The chemical and physical properties of PM was determined (Benito et al. 2006). It is can be assumed that applying PM into soil can improve soil chemical properties and biological properties, meanwhile because of the texture characteristics of PM, application of will improve soil physical properties as well (Hati et al. 2007).

However, the detail effects of PM application on soil properties are needed to further investigate, the appropriate application method of PM, and the usable range of application of PM were unclear. Therefore, in order to perfect the sustainable use of pruning materials as compost, clarify the detail effect of PM on soil, improving duration on plant organic matter content and microbial activity and effect on plant growth is necessary.

## 1.4 Present research state

### 1.4.1 The mineralization characteristics of pruning materials and PM

Because of the high texture characteristics of pruning materials, there is large amount of total C content in pruning materials (Kanbara et al. 2016). And because of mineralizable C is positive correlated with total C content (Toda and Haibara 1999), thus there is large amount of mineralizable C content in pruning materials. There is different content of mineralizable C in pruning materials from different species of trees (Kanbara et al. 2016). With process of composting of pruning materials, the organic matter content decreases gradually, and with organic matter content decreased the mineralizable C decrease gradually (Benito et al. 2003). In different composting stage, the mineralizable C content is different (Benito et al. 2005). Although the mineralizable C content decreased obviously comparing with the pruning materials, however comparing with conventional compost usually used in agriculture and green space, the detail mineralizable C content in PM and detail mineralization characteristics are unclear.

### 1.4.2 The effect of applying PM on soil organic matter content and microbial activity

In cultivated soil with different organic matter content and microbial activity, small amount of PM application such as $120 m^3 \cdot ha^{-1}$ and $240\ m^3 \cdot ha^{-1}$, through investigating, it can be found that, application of PM increased the soil the porosity and water-holding capacity of soils effectively. In addition, application PM into cultivated soil with low organic matter content and microbial activity, increased the soil CEC, cation base saturation, and microbial activity obviously (Takahashi et al. 2009, Liu et al. 2016). However, in soil with high organic matter content, the effect is not obviously. PM is nearly the same as that of conventional compost made from dung, leaves, or bark, on improving soil chemical properties as indicated by its cation exchange capacity, base cation saturation, and total carbon (C) and nitrogen (N) contents (Liu et al. 2016). Therefore, it can be inferred, application of PM will increase the soil organic matter content, however because of the different texture of PM comparing with other conventional compost, therefore the mineralization process of PM in soil is different with other compost (Chen et al 2014). However, the effect different amount of PM application on soil properties are unclear.

### 1.4.3 The effect of application of PM on plant growth

In master course, the experiment of planting oat in soil mixed with conventional compost (bark, dung and leaves) and PM had been conducted. Through experiment it can be found that, there is no obviously effect on plant growth with application of compost bark and leaves, however application of dung compost promoted plant growth obviously, application of PM into soil inhibited plant growth obviously (Liu, 2015).

### 1.4.4 Summary of present research state

Although through composting, the mineralizable C content reduce obviously (Benito et al. 2003), however it is always unclear that compared with conventional compost usually used in agriculture and green space, PM has more or less mineralizable C content. Application of PM into soils with low organic matter content and microbial activity, increased soil total C and N obviously. However, in soil with high organic matter content and microbial activity, the effect of small amount of PM on soil is not obviously. Therefore, the effect of PM application on soil with different organic matter content and microbial activity is different. However, the effect of different amount of PM application on soil properties is unclear. In addition, the effect duration of PM on promoting soil microbial activity is unclear either. In master course experiment I found that application of PM into soil inhibited plant growth when planting immediately (Liu 2015). However, the reason of inhibition on plant growth is unclear, and the inhibition duration is unclear, and the effect of PM on different kinds of plants is unclear.

## 1.5 The objective of the research

During process of green recycle, the organic matter mineralization is a very important step. Effect of Compost application on soil is accompanied with organic matter mineralization in compost thus the microbial activity is necessary to maintain the green recycle efficiently. Application of PM into soil, the nutrient release and soil properties change are started with PM mineralization. Because of the PM mineralization is affected by soil biological properties, it can be assumed that, the application effect of PM into soil with different biological properties is different.

The objective of this study is to propose how to use the compost made from pruning materials (PM) to maintain organic matter content and soil microbial activity meanwhile avoid negative effect on plant growth in soils with different organic matter content and microbial activity.

To propose the comprehensive use of PM into soil to improve soil organic matter and microbial activity meanwhile avoid the negative effect on plant growth, we need to understand the effect duration of PM on soil microbial activity, total C and N. in addition, we need to understand the detail effect of different amount of PM on soil with different organic matter content and microbial activity. Meanwhile we need to determine the effect of PM application on plant growth, when applying different amount of PM into soils with different organic matter content and microbial activity. Therefore, this study contains three part as below:

1. Determine the C mineralization characteristics of PM after mixing with soil through comparing with conventional compost and the carbon mineralization characteristics in different soils.
2. Determine the effect duration and detail effect of different amount of PM on promoting soil organic matter content and microbial activity.
3. Determine the effect of different amount of PM application on different plants growth and the effect on different plant growth in different phase after mixed with PM into soil.

## 1.6 The structure of this study

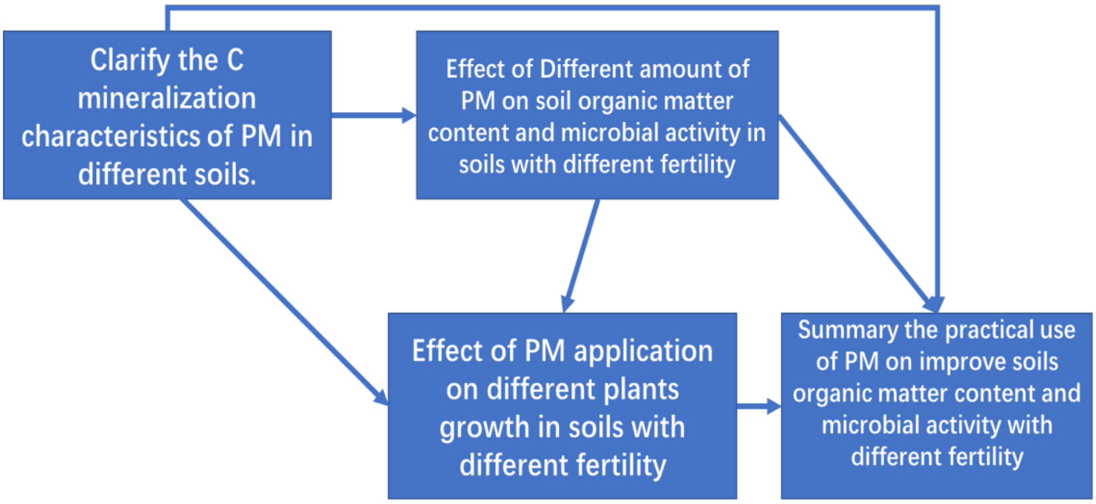


Fig 1-1 the process of the research

This study was conducted with the process as showed by Fig 1-1.

At first chapter 2: Through comparing with conventional compost usually used in agriculture and green space to determine the detail of C mineralization characteristics such as mineralizable C content, the mineralization rate and apparent energy. Then through incubate the PM in soils with different organic matter content and microbial activity, to clarify the C mineralization characteristics in soil with different organic matter content and microbial activity.

At second, chapter 3: Comparing with the conclusion from chapter 2, through the analyzing C mineralization characteristics, to estimate the effect of PM on plant growth in soils with different organic matter content and microbial activity. Through planting Leguminous plants and Cruciferae plants to determine if there is N starvation for plants caused by application of PM, and through a year planting of Komatsuna to determine the effect duration of PM on plant growth in soils with different organic matter content and microbial activity.

At third, chapter 3: Combine the conclusion of chapter 2 and 3, design the experiment of application of different amount of PM into soil with different organic matter content and microbial activity. To clarify the effect of amount of PM on soil with different organic matter content and microbial activity, meanwhile determine the effect duration of PM on improving soil organic matter and microbial activity in soil with different microbial activity. And investigate the dynamic of N and microbial biomass change after mixing with PM into soils with different organic matter content and microbial activity to determine the reason of inhibition caused by application of PM on plant growth in soil with low microbial activity.

At last, summary the practical use of PM on soils with different organic matter content and microbial activity in agriculture or in green space with different root deepness vegetations to determine the beneficial utilization of PM in chapter 5.

# Chapter 2 Carbon mineralization characteristics of compost made from pruning material

## Abstract

To investigate carbon (C) mineralization characteristics of composts made from pruning material (PM), C mineralization parameters were evaluated, including mineralizable C ($C_0$), apparent activation energy (*Ea*), and the rate constant of mineralization (*k*). These properties associated with conventional composts made from dung (D), fallen leaves (L), and bark (B) were also examined. On comparing *Ea* of different composts, no significant differences among the plant composts (L, B and PM) were observed. Notably, the values of the respective indicators were significantly greater than those of D. $C_0$ of PM was significantly greater than that of other composts. Conversely, the *k* value for PM was significantly smaller than that for other composts. These results indicated that PM supplies a substantial amount of mineralizable C, which persists for a long time after mixing with the soil. In addition, the PM mineralization rate in different soils is different, such as in forest soil the mineralization rate is faster than in subsoil. Therefore, it can be inferred that application PM into soil, mineralization process is shorter than subsoil, the mineralization process of PM in soil with high microbial activity is shorter.

**Key words: Mineralizable Carbon, Apparent activation energy, Rate constant of mineralization**

## 2.1 Introduction

According to a previous research, conventional compost, such as dung compost (D), leaf compost (L), and bark compost (B), is generally used in agriculture (Yamamoto et al. 2011), and the application of pruning materials compost (PM) can improve the chemical properties of soil (cation exchange capacity (CEC), salty base saturation, total carbon and nitrogen content, and pH), similar to the application of conventional D, L, and B (Liu et al. 2016). However, the mineralization characteristics of C in PM after mixing it with soil have not been extensively reported. The determination of the characteristics of C mineralization and their mechanisms after mixing PM into the soil can contribute to the effective prediction of C dynamics, which is accompanied by nutrient release and C storage of organic matter (Smith et al. 1993). Moreover, clarifying the C mineralization characteristics of PM is necessary to determine their effects on soil improvement. Many studies have assessed the amount of $CO_2$ emission from various composts to reflect the C mineralization characteristics (Pascual et al. 1998, Mamo et al. 1999). In addition of the texture of organic matter, the soil microbial activity also affect on the C mineralization during the process of PM feedback to soil (Hobbie 2015).

Therefore, in this study the mineralization characteristics of PM were investigated based on the emission of $CO_2$, with selected conventional composts D, L, and B used as comparative indicators. And through comparing the C mineralization characteristics in soil with different microbial activity to determine the C mineralization rate and mineralizable carbon content in different soils.

## 2.2. Materials and methods

### 2.2.1 Materials

#### 2.2.1.1 Composts

The compost types used in this experiment were as follows: D (Akimoto Tensanbutsu Corporation), L (Same as above), B (Same as above), and PM (Agora Landscape Architecture Corporation). The process of composting pruning materials was divided into two phases: an oxygen bio-oxidative phase that lasted for 30 days, in which 30% of PM was used as the microorganism source and aeration was performed once a week to provide sufficient oxygen for composting and the maturation phase that lasted for 100 days. The duration of the total composting process of PM was 130 days. Table 2-1 presents the total C and nitrogen (N) content, C:N ratio, and water content of each compost type, the total C, N, and C:N ratio of the composts were measured using a CN corder（YANACO Analytical Industry Co., Ltd.MT-700). The composts used in this experiment were crushed into powder (particle size <1 mm) using OSAKA Wonder Crusher (OSAKA CHEMICAL Co., Ltd.; WC-3).

**Table 2-1. Carbon (C) and Nitrogen (N) content in different compost materials (mean ± standard deviation)**

| Compost | Total C* (g·kg$^{-1}$) | Total N* (g·kg$^{-1}$) | C:N ratio** | Water* (%) |
|---|---|---|---|---|
| **D** | 332.5 ± 7.2 | 18.6 ± 0.55 | 17.87 | 70.20 ± 2.21 |
| **L** | 294.7 ± 3.7 | 17.2 ± 0.42 | 17.13 | 68.82 ± 3.12 |
| **B** | 382.0 ± 4.6 | 24.5 ± 0.31 | 15.59 | 39.45 ± 1.11 |
| **PM** | 401.2 ± 4.3 | 14.3 ± 0.57 | 28.05 | 53.02 ± 1.94 |

***Measured in different composts，**Only as the mean value.**

**D, dung compost; L, leaves compost; B, bark compost; PM, pruning material compost**

#### 2.2.1.2 Soil

The soil used in this experiment which determined the C mineralization characteristics of PM through comparing with conventional compost (Dung, leaves and bark compost) was collected from the surface soil layer (0–10 cm) in the forest and at the Matsudo Campus of Chiba University (Matsudo city, Chiba Prefecture, Japan) and passed through a 2-mm sieve. Table 2-2 presents the properties (water content, pH, total C and N contents, and C:N ratio) of the soil. The total C, N, and C:N ratio of the composts were measured using a CN corder（Same as above).

### 2.2.2 Incubation method

In total, 3 g of each dry-crushed compost sample was mixed with fresh soil with an equivalent dry weight of 30 g. To supply a sufficient amount of nitrogen for the propagation of soil microorganisms, we mixed a certain amount $NH_4Cl$ to obtain a C:N ratio of 15 for each compost sample (Table 2-3) (Kanbra et al. 2016). $NH_4Cl$ was added to the samples to shorten the incubation time; this did not change the mineralizable C content but only increased the mineralization rate (Henriksen and Breland 1999).

Water was added into the mixtures comprising soil and each compost type to establish a change in the water content of the newly mixed compost samples relative to their original water content. The samples were weighed once a week, and water was added as necessary to maintain a constant supply of water, as determined by the weight at the initial stages of the experiment.

**Table 2-2. Properties of soil used in the experiment**

**(mean ± standard deviation)**

| Soil properties | Value |
|---|---|
| Total C, ($g \cdot kg^{-1}$) | 25.50 ± 0.70 |
| Total N, ($g \cdot kg^{-1}$) | 1.75 ± 0.04 |
| C:N ratio* | 11.60 |
| pH | 6.9 ± 0.17 |
| Water content, (%) | 37.80 ± 2.20 |

***Only as the mean value**

**Table 2-3. Amount of $NH_4Cl$ added to obtain the desired C/N ratio (15) for each compost mixed with soil**

| Compost | Amount of $NH_4Cl$ added (g) |
|---|---|
| **Dung** | 7.70 |
| **Leaves** | 4.79 |
| **Bark** | 0.95 |
| **Pruning material** | 30.75 |

Each soil sample mixed with different compost types was transferred into a 500-mL glass jar. Fresh soil corresponding to 30g of dry soil weight was also placed into a glass jar to serve as the control blank. This experiment was performed in quadruplicate to verify the empirical data. The mixtures of soil and compost were incubated at three temperatures (20°C, 25°C, and 30°C) and analyzed for C mineralization parameters using the kinetics analysis model (Toda and Haibara 1999, Sugihara 1986).

The $CO_2$ emission levels were measured at intervals from 3 to 21 days. During incubation period, 10 measurements of $CO_2$ emission were obtained. The $CO_2$ emission from compost was estimated by calculating the difference between $CO_2$ emission from the compost mixed with the soil and the blank control. The rate of $CO_2$ emission was measured using the alkali absorption method (Naganawa 1992). The alkaline absorption method is operated as follows. 10ml of the 1cal $mol^{-1}$ KOH solution was filled into a 50ml beaker, and then the beaker was placed in a glass jar, followed by incubation. After each incubation, the KOH consumed by $CO_2$ was confirmed by titration with 1cal $mol^{-1}$ HCl. The $CO_2$ emission amount is calculated by the consumption of KOH, thereby calculating the $CO_2$ emission rate. The level of carbon mineralization is calculated by the $CO_2$ emission rate.

### 2.2.3 Numerical analysis of C mineralization parameters

C mineralization parameters were calculated based on an analysis model (Toda et al. 1997, Sugihara 1986). The parameters used in this research to determine the mineralization characteristics were as follows: mineralizable carbon content in the compost that can be utilized by microorganisms in the soil ($C_0$), apparent activation energy (cal·mol$^{-1}$) for C mineralization in compost (*Ea*), mean decomposition rate constant of C content in compost (day$^{-1}$; k), and percentage of mineralizable C to total C content in compost [$C_0/C_T$ (%)].

Equation (2) (Toda et al. 1997) was derived from Equation (1) (Inubushi 1994), where in the cumulative mineralized C in the compost was approximated with $C_0$ values obtained at the three experimental temperatures. The relationship between the mineralization rate constant and temperature follows the Arrhenius's law, as expressed by Equation (3) (Sakanoue et al. 1988, Sugihara 1986). $C_t$ is the C content after mineralization at time t; C is the cumulative amount of C mineralized at time t; $C_0$ is the mineralizable C; $k$ is the decomposition rate constant of C in different composts (day$^{-1}$); $C_0/C_T$ (%) is the percentage of mineralizable to total C content in the compost; t is the incubation time (days); *Ea* is the apparent activation energy (cal·mol$^{-1}$) for carbon mineralization in the composts; *R* is the gas constant (1.987 cal·deg$^{-1}$·mol$^{-1}$); T is the absolute temperature (K); and A is a constant.

$$C_t = C_0 e^{-kt} \qquad (1)$$

$$C = C_0 - C_t = C_0 \{1 - \exp(-k \cdot t)\} \qquad (2)$$

$$k = A \cdot \exp(-Ea / R \cdot T) \qquad (3)$$

To calculate *Ea* for composts, kinetics analysis (Sugihara 1986) was used, and based on Equation (3), Equation (4) was derived. Using Equation (4), the incubation time at 30°C and 20°C was used to calculate the incubation time at 25°C. Equation (5) was then be obtained by substituting t in Equation (2) with $t_{25°C}$. *Ea*, $k_{25°C}$, and $C_0$ in Equation (5) were calculated by the least squares method using the Solver function in

Excel 2010 (Microsoft Office 2010).

$$t_{25°C} = t \cdot \exp(-Ea \cdot \Delta T / R \cdot T \cdot 298) \qquad (4)$$

$$C = C_0 [1 - \exp\{k_{25°C} \cdot t \cdot \exp(-Ea \cdot \Delta T / R \cdot T \cdot 298)\}] \qquad (5)$$

Where $t_{25°C}$ is the incubation time at 25°C, ΔT is the difference between the different temperatures converted to 25°C, and $k_{25°C}$ is the decomposition rate constant at 25°C.

Due to the sharp changes in $CO_2$ emission rates during the early stage of incubation, the values of the parameters were unstable. Reliable parameter values were obtained only when the $CO_2$ emission rate considerably decreased compared with the initial rate. To obtain a reliable *k* value of C mineralization, the incubation time for the mineralization of half mineralizable C content was used as the least incubation time for C mineralization of composts. Sugihara (1986) proposed Equation (6) to calculate the least incubation time.

$$t_m = \ln(0.5) / k \qquad (6)$$

Where $t_m$ is the least incubation time (days) and *k* is the rate constant of mineralization.

As mentioned above, mineralizable C content in composts can be used by soil microorganisms. Therefore, the percentage of mineralizable C to total C in composts is an indicator for the activities of soil microorganisms.

### 2.2.4 Statistical analysis

To examine the difference in the parameters among the composts studied, one-way analysis of variance (ANOVA; multiple comparisons) was performed, followed by the least significant difference test. The results were considered significantly different at $p < 0.05$. All the values were presented as the mean. SPSS 19.0 program was used to perform all statistical analyses.

## 2.3. Results

The least incubation time was 8 days for D, 18 days for L, 13 days for B, and 39 days for PM. The total incubation time was 68 days, which was sufficient to obtain reliable data. Fig2-1 presents the changes in the cumulative value of mineralized C content in PM at the three examined temperatures. The cumulative content of mineralized C considerably increased with an increase in the incubation temperature.

After converting the incubation time at 20°C and 30°C into an incubation time at 25°C, the relationship between the incubation time and cumulative mineralized C of the composts was determined (Fig 2-2). The $CO_2$ emission rate of composts was relatively high initially, then decreased, and finally became stable over time. Compared with other composts, the $CO_2$ emission rate of PM became stable after a relatively long period of time, thus indicating a longer incubation period. The cumulative mineralized C content was within the range of 10–20 g C $kg^{-1}$ in D, L, and B but was within 50–60 g C $kg^{-1}$ for PM.

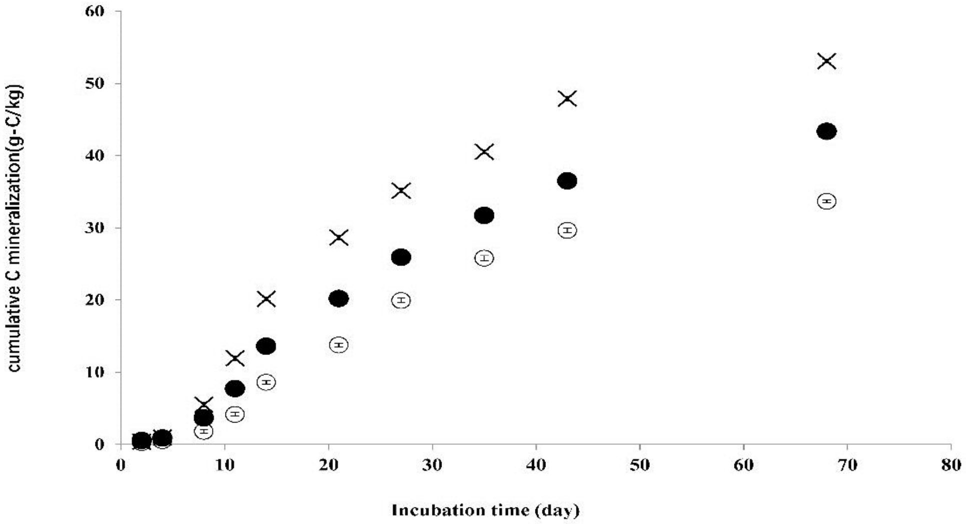


**Fig 2-1 Relationships between cumulative C mineralization and incubation time required to compost pruning materials (PM) under different temperatures. O: 20°C, ●: 25°C, ╳: 30°C**

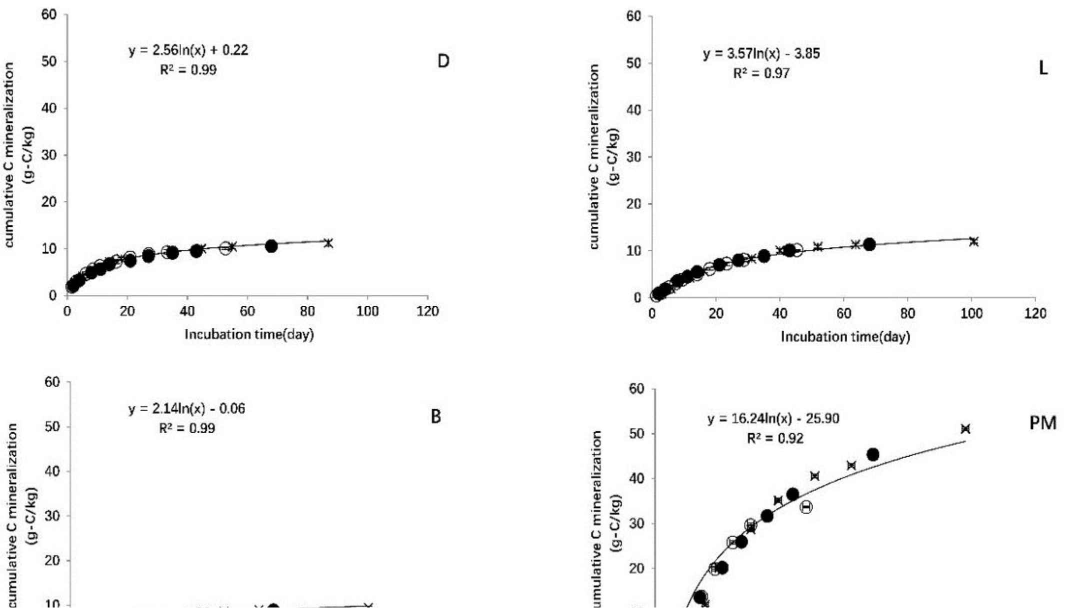


**Fig 2-2 Relationship between cumulative C mineralization and incubation time at 25°C. o: 20°C, ●: 25°C, ╳: 30°C**

Table 2-4 lists the C mineralization parameters of different composts, which indicated that $C_0$ of PM was significantly greater than that of conventional composts (D, L, and B). Conversely, $k$ of PM was significantly lower than those of other conventional composts (D, L, and B). Comparisons among the composts showed that $Ea$ values of composts derived from plants composts (L, B, and PM) were within the range of 53.2–58.9 kJ $mol^{-1}$, which were significantly higher than those of D. No significant differences were found among plant composts (L, B, and PM). $C_0/C_T$ (%) of PM was significantly greater than those of conventional composts (D, L, and B).

**Table 2-4. Carbon (C) mineralization parameters in different composts (mean ± standard deviation)**

| Compost | $C_0$* ($g \cdot kg^{-1}$) | $k$** ($day^{-1}$) | $E_a$*** ($kJ \cdot mol^{-1}$) | **C0/Total C** **** (%) |
|---|---|---|---|---|
| **D** | 10.11 ± 0.04a | 0.082 ± 0.005a | 37.10 ± 0.46a | 3.00a |
| **L** | 12.43 ± 0.82c | 0.038 ± 0.003b | 58.90 ± 1.23b | 4.20c |
| **B** | 8.44 ± 0.49b | 0.043 ± 0.002b | 57.80 ± 0.98b | 2.20b |
| **PM** | 61.81 ± 1.04d | 0.018 ± 0.001c | 53.20 ± 1.14b | 15.40d |

***Mineralizable C content**

****Rate constant of C mineralization**

*****Apparent activation energy for $CO_2$ generation**

******Only as the mean value**

**Different letters indicate significant differences at $p < 0.05$. The abbreviations (D, L, B and PM) are the same as table 1**

## 2.4. Discussion

The PM was supported by Agora Landscape Architecture Corporation, therefore the basic parameters of PM are different from different production, thus the basic parameters of PM in different experiment need to be measured one more time.

$C_0$ in PM was significantly greater than that in conventional composts (D, L, and B). Compared with the findings of López-González et al. (2013) and Benito et al. (2005), 130 days are sufficient for the composting of PM , after which the PM can be considered mature. Compared with L, PM mainly comprises branches and trunks, which are rich in xylem tissue and have a complex composition. Furthermore, it contains many tracheid cells, which comprise single cells with the cellulose wall thickened with numerous pits of lignin in the xylem (Denning et al. 2005). Due to the complex texture and large content of cellulose and hemicellulose mixed with lignin in PM, it contains a larger amount of mineralizable C after composting. Thus, the mineralizable C content in PM is greater than that in conventional composts obtained from D and L. The relative lignin content in compost B is significantly lager than that in branches (Nawawi et al. 2016). Lignin is an integral cell wall constituent that provides strength and resistance to microbial degradation (Tuolema et al. 2000). During the composting of plant material and mineralization of the compost in soil, cellulose and hemicellulose are degraded with almost no lignin degradation (López-González et al. 2013, Gani and Naruse 2017, Weintraub and Schimel 2003). In addition to the high lignin content in B, we believe that the larger particle size of wood chips in pruning materials limits the access of microorganisms to mineralizable C during composting, which is the main factor responsible for the high mineralizable C content in PM. Therefore, the mineralizable C content in PM is greater than that in B. Because of the large mineralizable C content and complex structure of PM that makes the evaluation of soil microorganisms difficult to contact with cellulose and hemicellulose, the value of *k* in PM was significantly lower than that in its conventional counterparts (D, L, and B). Moreover, no significant differences were observed in *Ea* values among the plant composts (L, B and PM), although it was significantly higher in PM than in D. In addition, the parameters vary according to the type of the compost. For example, D mainly comprises proteins and lipids, whereas plant-derived compost predominantly contains cellulose and hemicellulose. Taken together, our results suggested that the sources of $CO_2$ in plant (L, B, and PM) and animal (D) were responsible for the difference in *Ea* values.

In conclusion, PM contains larger amounts of $C_0$ but is characterized by lower $k$ values than those of conventional composts (D, L, and B). Microbial activity is enhanced for a long time after the application of PM in the soil. However, the application of composts with large amounts of $C_0$ and high C:N ratios may cause N starvation (Teutscherova et al. 2017). Therefore, further investigation of the effect of PM application on plant growth is required to optimize its use as an effective method for improving the soil quality.

## 2.5. Conclusion

Comparing with conventional compost, Application of pruning materials compost

(PM) into soil, supply more mineralizable C as resource to microorganisms thereby maintain microbial activity in soil for longer time. Meanwhile comparing with characteristics of mineralization of PM in forest soil and subsoil, mineralize rate of PM in forest soil is faster than in subsoil. Therefore, the phase of PM mineralization in soil with high microbial activity is shorter.

# Chapter 3 The effect of application of PM on different plants in different soils

## Abstract

Compost prepared using pruning material (PM) contains a higher amount of mineralizable carbon (C) than conventional compost, readily causing nitrogen (N) immobilization in soil due to the multiplication of microorganisms and subsequently likely causing N starvation for plant growth inhibition. However, organic matter mineralization in soil is affected by soil microbial activity, which correlates with the total C and N contents. Therefore, we hypothesized the application of PM to different organic matter content and microbial activity soils have different effects on plant growth depending on soil microbial activity. Through comparing with effect of application of PM on plant growth of komatsuna (*Brassica rapa* L. *var. perviridis* LH Bailey) and kidney bean (*Phaseolus vulgaris* L.) in different soils with equal applications of PM for 1 month. We found that PM application significantly inhibited plant growth of Komastuna in the soil with low microbial activity, but significantly accelerated plant growth of Komatsuna in the soil with high microbial activity suggesting that different effects are observed on Cruciferae plant growth in soils with different microbial activities. However, there is no inhibition occurred on plant growth of leguminous plants. In conclusion, these findings indicate that the application of PM to soil can accelerate plant growth in soils with high microbial activities but can inhibit plant growth except leguminous plants in soils with low microbial activities. Therefore, it can be inferred that except for nitrogen starvation in soil with low microbial activity, there is no negative effect of application of PM on plant growth and planting plants with capacity of N fixation can avoid the N starvation. With time passed, the effect of N starvation on inhibiting plants disappeared gradually.

**Key word: PM; plant growth; inhibition; promotion**

## 3.1. Introduction

PM contains high concentrations of cellulose and hemicellulose with a large amount of lignin, and it consequently has a higher mineralizable C content than conventional compost (Liu et al. 2019). Consequently, its application may have a priming effect on organic matter mineralization in soil (Zimmerman et al. 2011; Liu and Takahashi 2019), which can have positive and negative effects on soil properties (Dempster et al. 2012). For example, although its application can improve the chemical, physical, and biological properties of soil, it can also cause N immobilization (Dempster et al. 2012). Thus, while mixing PM with soil can effectively increase in soil organic matter content, a high mineralizable C content may inhibit plant growth due to N starvation (Benito et al. 2005) as a result of a rapid increase in microbial biomass (Thiessen et al. 2013). Therefore, it is important to determine the effect of application of PM on plant growth in different soil.

The objective of this study was to evaluate the effect of PM on microbial plant biomass in different soils with different microbial activities. Accordingly, we applied PM to soils with different microbial activities, grew komatsuna (*Brassica rapa* L. var. *perviridis* LH Bailey) and kidney bean (*Phaseolus vulgaris* L.) in these PM-supplemented soils, and measured the dry weight of each plant as an indicator of plant biomass.

## 3.2. Materials and method

### 3.2.1 Soil and compost

To investigate how the application of PM affect plant growth in different soils, a planting experiment was conducted using forest soil, subsoil, and sand (Table 3-1). The forest soil (0–10 cm depth) was collected from evergreen broad-leaved forest growing on the Matsudo Campus of Chiba University (Matsudo, Chiba Prefecture, Japan) and the subsoil (>30 cm depth) was collected from an experimental field on the Matsudo Campus of Chiba University. The sand was purchased. Different types of soils were produced by mixing varying proportions of forest soil, subsoil, and sand (Table 3-2), with the resulting soil types A to G representing high to low total C and N contents and microbial activities as determined by fluorescein diacetate (FDA) hydrolysis (Ichikawa et al. 2008) (Table 3-3). The compost was made from PMs obtained from Agora Landscape Architecture Corporation (Tokyo, Japan). The composting process was divided into two stages. In the first stage, 30% volume of PM as microorganism source was mixed with fresh wood chips (particle size < 25 mm) made from the PMs and was kept for 30 days, airing the mixture once per week. In the second stage, the wood chips mixed with PM were kept for a further 100 days for maturation. López-González et al. (2013) and Benito et al. (2005) reported that PM were composted sufficiently after 120 days. Therefore, the compost used in this experiment, which had been composted for 130 days, should have been sufficiently composted.

**Table 3-1. Properties of the soils and compost used in this experiment**

| Soil/compost | Water content (kg/kg) | Dry density (kg/L) | Fresh density (kg/L) | Total carbon content (g/kg) | Total nitrogen content (g/kg) | C/N ratio |
|---|---|---|---|---|---|---|
| **Forest soil** | 0.28 | 0.57 | 0.79 | 110.4 | 8.50 | 12.98 |
| **Subsoil** | 0.25 | 0.74 | 0.98 | 37.0 | 2.10 | 17.61 |
| **Sand** | 0.09 | 1.21 | 1.33 | 2.6 | 0.20 | 13.00 |
| **Pruning materials** | 0.44 | 0.17 | 0.3 | 396.0 | 16.30 | 24.29 |

**Table 3-2. Composition of the different soil types use**

| Soil | Experimental materials | Volume ratio |
|---|---|---|
| A | Forest soil | 1 |
| B | Forest soil:subsoil | 1:1 |
| C | Forest soil:subsoil | 1:2 |
| D | Subsoil | 1 |
| E | Subsoil:sand:PM | 1:1 |
| F | Subsoil:sand | 1:2 |
| G | Sand | 1 |

**PM: compost made from pruning materials.**

**Table 3-3 Total carbon (C) and nitrogen (N) contents and microbial activities of the different soils used**

| Soil | Total C (g/kg) | Total N (g/kg) | C/N ratio | FDA† | Water content (%) |
|---|---|---|---|---|---|
| A | 110.4 ± 9.01a | 8.5 ± 0.73a | 12.99 | 1.40 ± 0.044a | 27.78 |
| B | 74.6 ± 6.09b | 5.6 ± 0.49b | 13.32 | 1.20 ± 0.04b | 26.66 |
| C | 65.4 ± 5.34c | 5 ± 0.43c | 13.08 | 0.85 ± 0.028c | 26.85 |
| D | 37 ± 3.02d | 3.1 ± 0.24d | 11.94 | 0.66 ± 0.022d | 24.98 |
| E | 12 ± 0.98e | 0.9 ± 0.08e | 13.33 | 0.29 ± 0.01e | 15.51 |
| F | 5.1 ± 0.42f | 0.6 ± 0.03f | 8.50 | 0.21 ± 0.007f | 12.80 |
| G | 2.6 ± 0.21g | 0.2 ± 0.01g | 13.00 | 0.19 ± 0.009f | 8.53 |

**Different lower-case letters within a column indicate significant differences among soil types ($p$ < 0.05).**

### 3.2.2 Plant and pot

Rectangular pots (volume: 2.2 L; length: 25 cm; width: 11 cm; depth: 8 cm) were filled with soils and PM as treatments or only soils as a control. Each of the soil types were mixed with different ratios of PM, as shown in Table 3-4

**Table 3-4. Details of the treatments used in experiment**

| Soil† | Volume of PM added to soil (%) | Treatment | Volume ratio |
|---|---|---|---|
| **A** | 0 | Forest soil | 100% |
| | 7.5 | Forest soil:PM | 70%:30% |
| | 15 | Forest soil:PM | 85%:15% |
| | 30 | Forest soil:PM | 92.5%:7.5% |
| **B** | 0 | Forest soil:subsoil | 50%:50% |
| | 7.5 | Forest soil:subsoil:PM | 35%:35%:30% |
| | 15 | Forest soil:subsoil:PM | 42.5%:42.5%:15% |
| | 30 | Forest soil:subsoil:PM | 46.25%:46.25%:7.5% |
| **C** | 0 | Forest soil:subsoil | 33.3%:66.7% |
| | 7.5 | Forest soil:subsoil:PM | 23.3%:46.7%:30% |
| | 15 | Forest soil:subsoil:PM | 28.33:56.67:15% |
| | 30 | Forest soil:subsoil:PM | 30.83:61.67:7.5% |
| **D** | 0 | Subsoil | 100% |
| | 7.5 | Subsoil:PM | 70%:30% |
| | 15 | Subsoil:PM | 85%:15% |
| | 30 | Subsoil:PM | 92.5%:7.5% |
| **E** | 0 | Subsoil:sand:PM | 50%:50% |
| | 7.5 | Subsoil:sand:PM | 35%:35%:30% |
| | 15 | Subsoil:sand:PM | 42.5%:42.5%:15% |
| | 30 | Subsoil:sand:PM | 46.25%:46.25%:7.5% |
| **F** | 0 | Subsoil:sand | 33.3%:66.7% |
| | 7.5 | Subsoil:sand:PM | 23.3%:46.7%:30% |
| | 15 | Subsoil:sand:PM | 28.33:56.67:15% |
| | 30 | Subsoil:sand:PM | 30.83:61.67:7.5% |
| **G** | 0 | Sand | 100% |
| | 7.5 | Sand:PM | 70%:30% |
| | 15 | Sand:PM | 85%:15% |
| | 30 | Sand:PM | 92.5%:7.5% |

**† A: forest soil alone; B: 1:1 ratio of forest soil:subsoil; C: 1:2 ratio of forest soil:subsoil; D: subsoil alone; E: 1:1 ratio of subsoil:sand; F: 1:2 ratio of subsoil:sand; G: sand alone.**

**PM: compost made from pruning materials.**

### 3.2.2.1 Komatsuna planting for one year

We used Komatsuna and in this experiment. The experiment was carried out in rectangle pot. The volume of each pot is 2.2L, length: 25cm, wide: 11cm and depth: 8cm. 12 seeds of Komatsuna (Sakata Seed Corporation) were planted in each pot. After planted, the watering was continued once a day during planting period. And the planting period continued for one year. The Komatsuna planting experiment started at 20th August 2016, 20$^{th}$ March 2017 and 20$^{th}$ August 2017 and ended at 20$^{th}$ September 2017 continue one year for three times planting. The Komatsuna was harvested at 20$^{th}$ September 2016. After harvested, root and leaves of each individual plant were washed by water, then were put into paper bag, and through drying by oven, at last measured dry weight of each individual plant.

### 3.2.2.2 Kidney bean planting

The Kidney bean (*Phaseolus vulgaris* L.) was planted with the same method with planting Komastuna, however the pot component is same with the A, B, D, E, and G used in planting Komatsuna. The planting experiment was started at 2$^{nd}$ September 2017 and ended at 2$^{nd}$ October, 2017. After harvested, root and leaves of each individual plant were washed by water, then were put into paper bag, and through drying by oven, at last measured dry weight of each individual plant.

### 3.2.3 Statistical analysis

To examine whether PM application had a different effect on plant growth depending on the soil type, one-way analysis of variance (multiple comparisons) was performed followed by the least significant difference test. The correlation was measured using Pearson's correlation method. All statistical analyses were performed using SPSS 19.0 with a significance level of $P < 0.05$.

## 3.3. Result

### 3.3.1 The effect of application of PM on Komatsuna growth

Brassicaceae Plant growth was indicated by Komatsuna growth. Growth of the komatsuna plants, as indicated by the dry weights of individual plants, the result of planting Komatsuna for one year is show in Fig 3-1.

Through comparing with the three times of Komatsuna planting in soils with different amount of PM application in Fig 3-4, it can be found that applying PM into in soil with different organic matter content and microbial activity for one year.

At first time planting, in soils A and B, the application of 7.5% and 15% (v/v) PM significantly promoted plant growth ($p < 0.05$), while in soil C, only 7.5% PM had a significant effect ($p < 0.05$), with 30% PM inhibiting plant growth. In soils D to G, all levels of PM significantly inhibited plant growth ($p < 0.05$). At second time planting, in soils A to F, the application of 7.5% PMs significantly promote plant and 15% PM significantly promoted plant growth ($p < 0.05$), while in soil C, only 7.5% PM had a significant effect ($p < 0.05$), with 30% PM inhibiting plant growth. In soils D to G, all levels of PM significantly inhibited plant growth ($p < 0.05$).

At the second time planting, except in soil G, application 7.5% (v/v) of PM promoted plant growth in all soils ($p < 0.05$). However only in soil with high microbial activity such as soil A, B, C and D, application of 15% (v/v) PM promoted Komatsuna

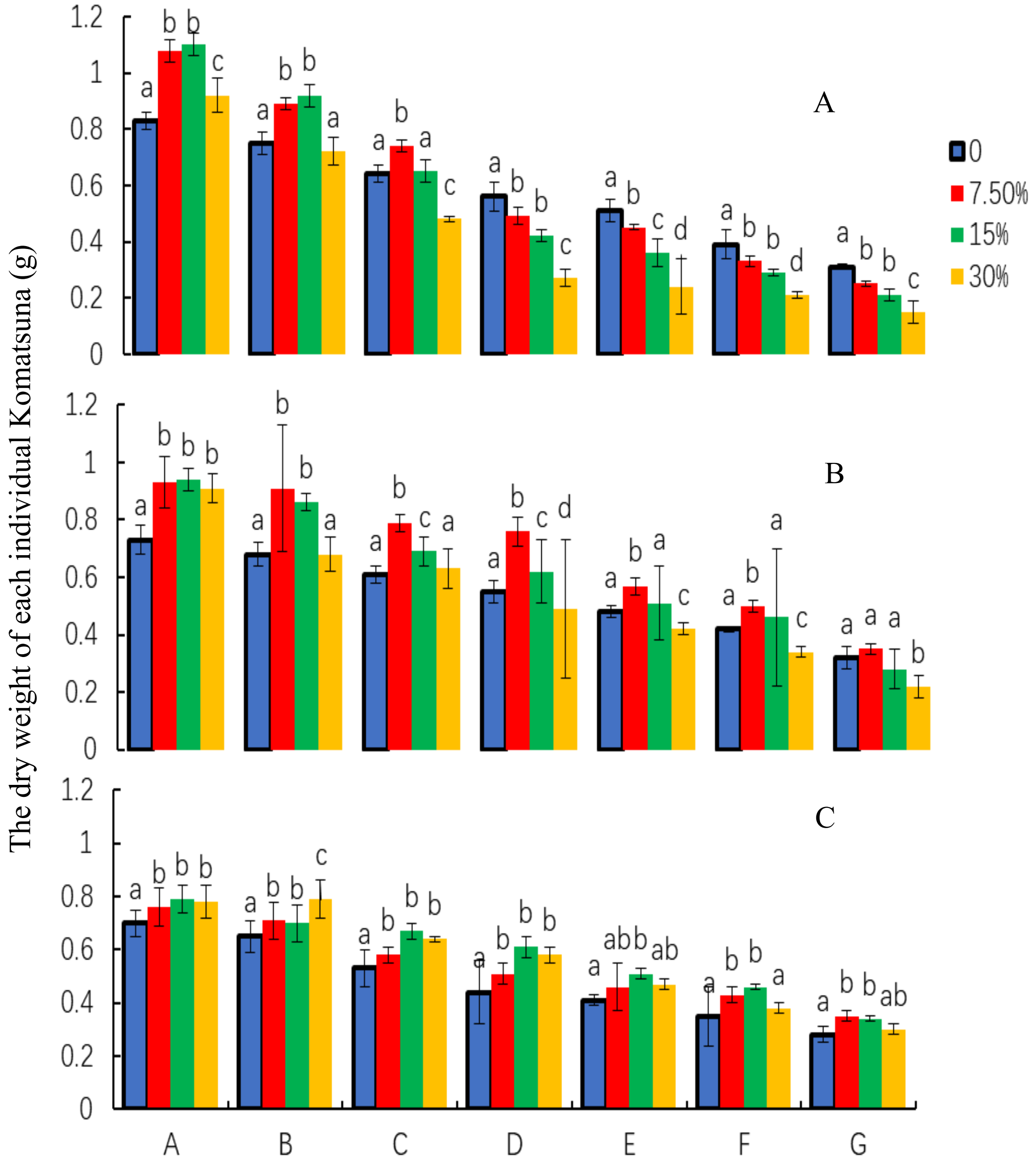


**Fig 3-1 Dry weight of each Komatsuna plant through a year planting. A: planted after mixed with PM, B: 6 months after mixed with PM, C: one year after mixed with PM.**

**Different lower-case letters within a column indicate significant differences among different concentration of application of PM into soils ($p$ < 0.05).**

growth ($p < 0.05$). In soil with low microbial activity such as soil E, F and G, application of 15% (v/v) PM haven't affected on Komatsuna growth. Only in soil A, application of 30% (v/v) PM promoted Komatsuna growth. In soil D, E, F and G, application of 30% (v/v) PM inhibited Komatsuna growth ($p < 0.05$).

At the third time planting, except in soil A, application 7.5% (v/v) of PM promoted plant growth in all soils ($p < 0.05$). In all soils, application 15% (v/v) of PM promoted plant growth in all soils ($p < 0.05$). And except in soil E, F and G, application of 30% (v/v) PM promoted Komatsuna growth.

Through comparing with the three times of Komatsuna planting in soils with different amount of PM application in Fig 3-4, it can be found that the promoted range of different soil is increasing. And the inhibition range is decreasing.

The ratio of the dry weight of a particular treatment group to the dry weight of the corresponding control group was used as an indicator of the effect of PM application on plant growth, whereby a ratio of >1 indicates plant growth promotion and a ratio of <1 indicates plant growth inhibition (Fig 3-2).

### 3.3.2 The effect of application of PM on Leguminous plant

The effect of application of PM on Leguminous plant was shown at Fig 3-3. Except in soil G, application of 7.5% (v/v) PM promoted the plant growth ($p < 0.05$), in soil A and B, application of 15% (v/v) PM promoted plant growth ($p < 0.05$). there is no inhibition occurred in any soil with any amount of PM application.

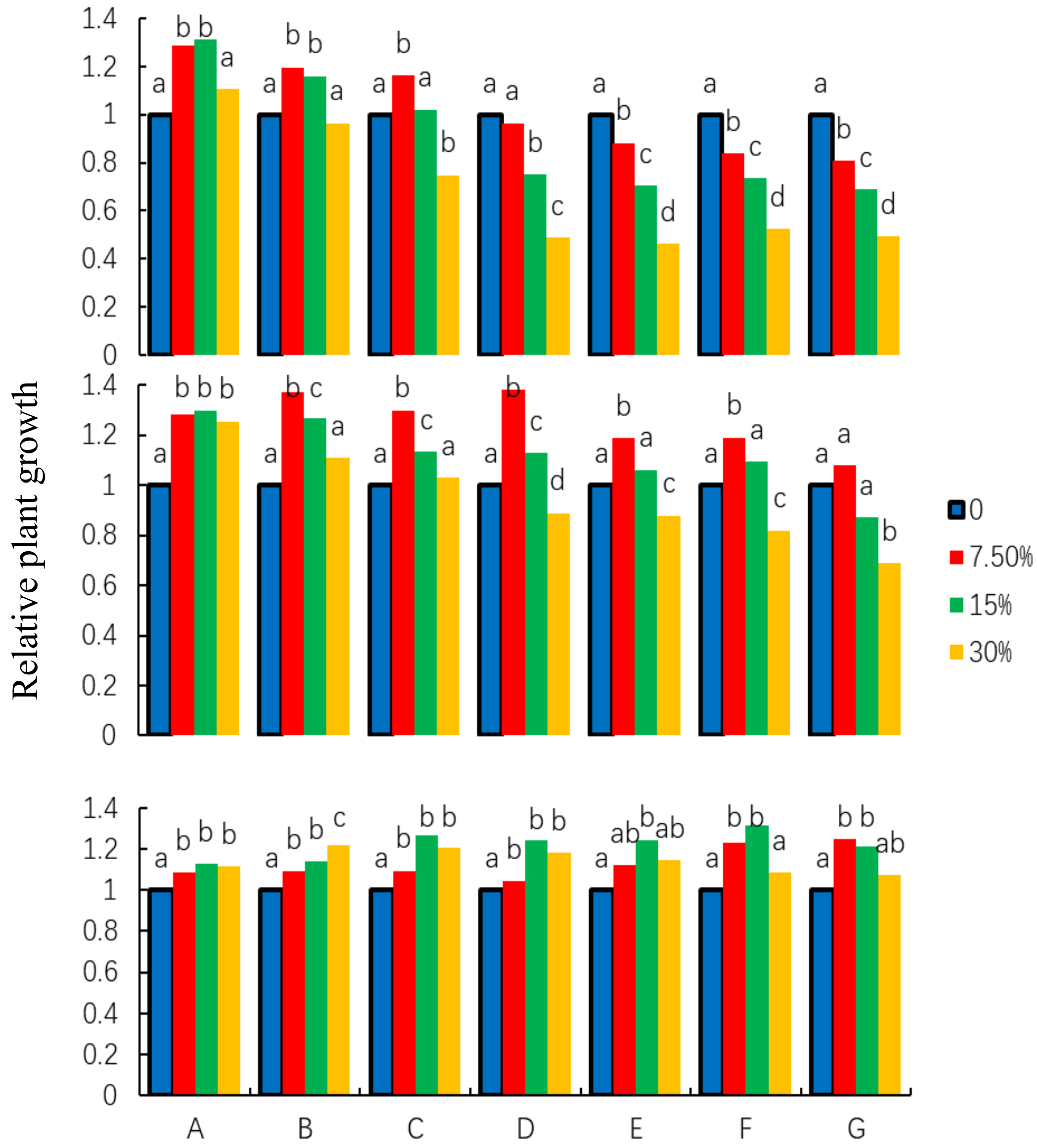


**Fig 3-2. Relative growth of komatsuna (*Brassica rapa* var. *perviridis*) plants in each treatment group compared with its corresponding control group. Four concentrations of compost made from pruning materials (PM) were applied to seven different soil types: (A) forest soil alone, (B) 1:1 ratio of forest soil:subsoil, (C) 1:2 ratio of forest soil:subsoil, (D) subsoil alone, (E) 1:1 ratio of subsoil:sand, (F) 1:2 ratio of subsoil:sand, and (G) sand alone. Relative plant growth was calculated as the ratio of the dry weight of an individual plant in a particular treatment group compared with the corresponding control group. * Significant difference compared with the control group ($p \leq 0.05$).**

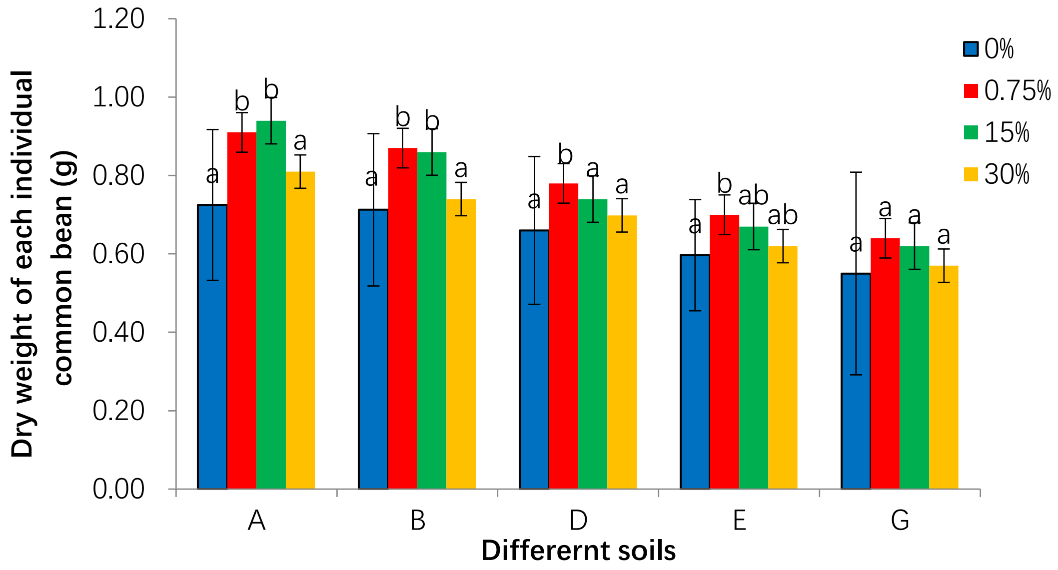


**Fig 3-3. Dry weight of Kidney bean individual in different treatment.**

**Different lower-case letters within a column indicate significant differences among different concentration of application of PM into soils ($p < 0.05$)**

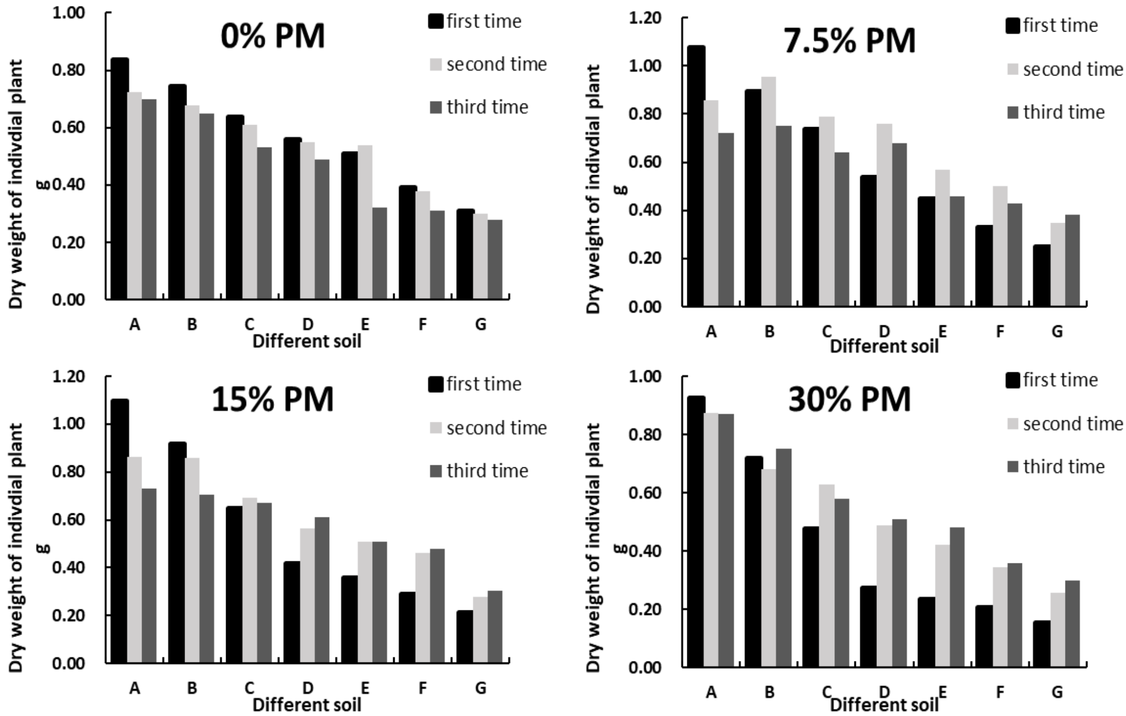


**Fig 3-4. The dry weight of individual plant in different treatments with different concentration of PM applying.**

## 3.4. Discussion

Comparing with the effect of PM application on plant growth between Komatsuna (Fig 3-1) and Kidney bean (Fig 3-3), it can be found that, in soil with low microbial activity, there is inhibition on Komatsuna growth, otherwise there is no inhibition on Kidney bean growth. Because of the Kidney bean belong to Leguminosae plant which has the capacity of fixing nitrogen from air, therefore the N starvation did not inhibit the plant growth of Kidney bean. Therefore, in soil with low microbial activity, there is N starvation occurred in soil with application of PM. However, because of there is no inhibition occurred in planting Kidney bean experiment, it can be found that there is no other negative effect on plant growth except N starvation.

The application of 7.5% PM significantly promoted plant growth in soils A–C, and 15% PM also significantly promoted plant growth in soils A and B (Fig 3-1). However, plant growth was significantly inhibited by the application of 7.5%, 15% PM in soil types D–G and 30% PM in all seven soil types. Compost contains numerous of microorganisms (López-González et al. 2015) and PM contain a large amount of mineralizable carbon (chapter 2), which increases soil microbial activity (Liu et al. 2019). Consequently, the application of PM increased microbial activity in the soil (chapter 4), which resulted in the consumption of inorganic N in the soil (Zimmerman et al. 2011) and lead to N starvation (chapter 4). Therefore, because of the long phase of N starvation caused by application of PM in soil with low microbial activity (chapter 4), PM application inhibited plant without capacity of N fixation growth in soil with low organic matter content and microbial activity.

The relationship between soil original microbial activity and relative plant growth was shown in Fig 3-5. There was a positive correlation between the original soil microbial activity and relative plant growth. The application of PM to soil that already had a relatively high microbial activity (soils A and B) had a promoted effect on soil microbial activity because more microorganisms were present (Table 3-3). There is a positive relationship between net N mineralization and N uptake by plants and application of PM in soil with high microbial activity (Kaye and Hart 1997), the high mineralization rate enhances nutrient release (Smith et al. 1993). Consequently, in soils A and B, the high original microorganism content in the soil contributed to the shortened process of organic matter turnover in soil and the reduced time until the net mineralized N was released from the applied PM to the soil, thus increasing plant N uptake and enhancing nutrient release. Therefore, the application of 7.5% and 15% PM in soil with high microbial activity increased net N mineralization and provided the necessary nutrients for plant growth, resulting in the immediate promotion of plant growth. However, the application of 30% PM added excess mineralizable C and microorganisms to the soil, resulting in the consumption of N being higher than the amount supplied. Consequently, the application of 30% PM did not significantly promote plant growth in soils A and B, and appeared to inhibit plant growth in soil C.

Therefore, it can be found that after mixed PM into soil, there is a turnover process. The turnover process of PM is correlated with soil original microbial activity (chapter 4). As fig 3-2 shows, more PM applied into soil, the relative plant growth is smaller. Therefore, during the turnover process of PM in soil, the consumption of N is correlated with application amount. Therefore, although application PM into soil can improve soil properties rapidly and obviously, however it will inhibit plant growth more obviously.

During the process of PM turnover in soils, the inorganic change is correlated with the microbial biomass change (chapter 4). The microbial biomass increasing first with application of organic matter (Hobbie 2015), thus PM application lead to microbial biomass increasing, therefore consume the inorganic N and organic N first, then with the microbial biomass mineralization, the N released with form of inorganic N (Marinari et al. 2010). Therefore, the turnover process of PM in soil is accompanied with the microbial biomass accumulating and mineralizing. And the microbial activity is positive correlated with the microbial biomass (Ichikawa et al. 2008). Therefore, the most obviously effect on plant growth with application of 7.5% (v/v) of PM was occurred at first time or second, and the most obviously effect on plant growth with application of 15 and 30 % (v/v) of PM was occurred at second time and third time (Fig 3-4).

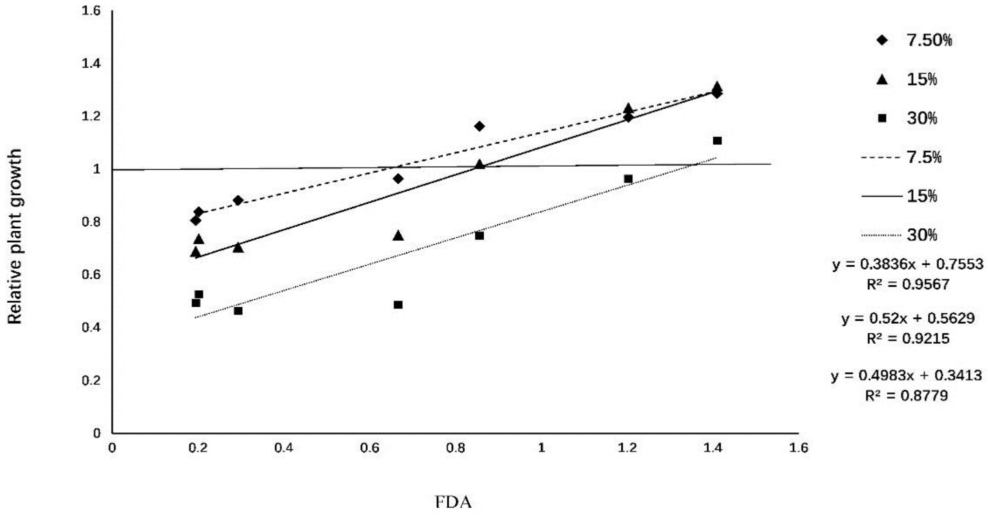


**Fig 3-5 Relationship between microbial activity and relative plant growth. The fluorescein diacetate (FDA) value is shown as the amount of FDA hydrolyzed per 1 g dry weight of sample. Relative plant growth was calculated as the ratio of the dry weight of an individual plant in a particular treatment group to the corresponding control group at first time planting.**

## 3.5. Conclusion

Because PM contain a large amount of mineralizable C, its application to the soil can readily cause N starvation due to the increased microbial activity. During the early stage of PM application, the addition of PM effectively promoted plant growth in soils with high microbial activity enough. Furthermore, the application of PM to soils with low microbial activity, lead to N starvation thus inhibited plant growth during the early stage, with this effect becoming more pronounced with increasing amounts of PM. And through comparing with plants with capacity of N fixation or not with PM application, there is no other inhibition on plant growth except for N starvation, therefore in addition to N starvation, there is no other negative effect on plant growth. With time passed, the inhibition of PM application on plant growth disappeared gradually, and the more PM application caused longer inhibition process and more obviously.

# Chapter 4 Effect of pruning material compost on the soil chemical and biological properties

## Abstract

Compost prepared using pruning material (PM) contains a higher amount of mineralizable carbon (C) than conventional compost, readily causing nitrogen (N) immobilization in soil due to the multiplication of microorganisms and subsequently likely causing N starvation for plant growth inhibition. However, organic matter mineralization in soil is affected by soil microbial activity, which correlates with the total C and N contents. Therefore, we hypothesized the application of PM to different organic matter content and microbial activity soils have different effects on soil properties depending on soil microbial activity. Using an incubation experiment, we found that the application of PM had a priming effect in forest soil and subsoil, causing N immobilization in both soils. However, the period of N immobilization depended on soil microbial activity, being shorter when the soil microbial activity was higher. To test the effects of PM on total C, N and microbial activity for 1 year. We found that PM application increased soil total C, N and microbial activity in soil with low organic matter content and microbial activity obviously no matter application amount. In soil with high organic matter content and microbial activity, PM application increased soil total C and microbial activity. In soil with high organic matter content and microbial activity, the reduce of total C and microbial activity, is focused on first 6 months, in soil with low organic matter content and microbial activity, reduce of total C and microbial activity is focused on later 6 months. The reduce of microbial activity is

positive correlated with reduce of total C.

**Key word: Soil microbial activity; N starvation; C microbial biomass**

## 4.1. Introduction

PM contains high concentrations of cellulose and hemicellulose with a large amount of lignin (Nawawi 2016), and it consequently has a higher mineralizable C content than conventional compost (Liu et al. 2019). Consequently, its application may have a priming effect on organic matter mineralization in soil (Zimmerman et al. 2011; Liu and Takahashi 2019), which can have positive and negative effects on soil properties (Dempster et al. 2012). For example, priming effect on soil organic matter and organic matter in PM can improve nutrients feedback, it can also cause N immobilization (Dempster et al. 2012). Thus, while mixing PM with soil can effectively increase in soil organic matter content, a high mineralizable C content may inhibit plant growth due to N starvation (Benito et al. 2005) as a result of a rapid increase in microbial biomass (Thiessen et al. 2013). Therefore, it is important to determine the N dynamic, microbial biomass change in soil which PM has been applied to optimize its effectiveness in improving soil quality.

The C and N cycling dynamics of compost-supplemented soils can be affected by compost feedstock, processing conditions, and mineralization time (Cambardella et al. 2003). In particular, compost feedback is affected by soil microbial activity which in turn, is affected by soil organic matter content and microbial activity gradients such as C and N availability (Riffaldi et al. 1996; Hobbie 2015); these positively correlate with the total C and N contents of soil (Toda and Haibara 1999). Therefore, we hypothesized that the application of PM will have different effects on total C and N contents and soil microbial activity and N dynamic in soil with different organic matter content and microbial activity.

The objective of this study was to evaluate the effect of PM on total C, N and microbial activity meanwhile the dynamic of microbial biomass, inorganic N concentration. Accordingly, we applied PM to soils with different microbial activities. We monitored the changes in microbial biomass C and inorganic N concentration by conducting an incubation experiment.

## 4.2. Materials and method

### 4.2.1. Soil and compost

To investigate how the application of PM affect plant growth in different soils, a planting experiment was conducted using forest soil, subsoil, and sand (Table 3-1). The forest soil (0–10cm depth) was collected from evergreen broad-leaved forest growing on the Matsudo Campus of Chiba University (Matsudo, Chiba Prefecture, Japan) and the subsoil (>30cm depth) was collected from an experimental field on the Matsudo Campus of Chiba University. The sand was purchased. Different types of soils were produced by mixing varying proportions of forest soil, subsoil, and sand (Table 3-2), with the resulting soil types A to G representing high to low total C and N contents and microbial activities as determined by fluorescein diacetate (FDA) hydrolysis (Ichikawa et al. 2008) (Table 3-3). The compost was made from PMs obtained from Agora Landscape Architecture Corporation (Tokyo, Japan). The composting process was divided into two stages. In the first stage, 30% volume of PM as microorganism source was mixed with fresh wood chips (particle size < 25 mm) made from the PMs and was kept for 30 days, airing the mixture once per week. In the second stage, the wood chips mixed with PM were kept for

a further 100 days for maturation. López-González et al. (2013) and Benito et al. (2005) reported that PM were composted sufficiently after 120 days. Therefore, the compost used in this experiment, which had been composted for 130 days, should have been sufficiently composted.

### 4.2.2 Total C and N contents, C/N ratio, and microbial activities in the soils and PM

Rectangular pots (volume: 2.2L; length: 25cm; width: 11cm; depth: 8cm) were filled with soils and PM as treatments or only soils as a control. Each of the soil types were mixed with different ratios of PM, as shown in Table 3-4. The samples were sellected at 20th August 2016, 20$^{th}$ March 2017 and 20$^{th}$ August 2017 continue one year for three times. The samples were collected for measuring total C, N, C/N ratio and microbial activity.

The total C and N contents and C/N ratio of the soils and compost were analyzed using a measured using a CN corder（YANACO Analytical Industry Co., Ltd.MT-700). Microbial activity was measured using the FDA hydrolysis method (Ichikawa et al. 2008). The FDA value used in this experiment was based on the amount of FDA hydrolyzed per 1g dry weight of each sample.

### 4.2.3 Microbial biomass C and inorganic N

The microbial biomass C and inorganic N concentrations were measured for four combinations of the soils and PM: soil F only, soil S only, soil F mixed with PM (1:3 fresh weight ratio), and soil S mixed with PM (1:3 fresh weight ratio).

2000ml glass jars were filled with soils F and S representing control groups and with soil F mixed with PM and soil S mixed with PM representing treatment groups. The weight of soil in each jar was 400g. The soils were incubated in an incubator at 25℃ for 80 days, during which samples were collected on days 1, 7, 22, 36, 50, and 80 for the measurement of microbial biomass C and inorganic N concentrations.

Microbial biomass C was measured using the fumigation-extraction method (Vance et al. 1987). The effect of PM application on microbial biomass C was reflected by an increment in microbial biomass C in the treatment groups, which was calculated using equation (1):

Increment in microbial biomass C = microbial biomass $C_{(soil\ with\ PM)}$ − microbial biomass $C_{(soil)}$ ---------- (1)

Inorganic N concentration in the soils was determined by measuring the amounts of nitrite N and ammonium N. From each glass jar, 10 g sample of wet soil was collected and extracted using 50mL of 1M potassium chloride solution. Ammonium concentration in each extract was determined using the modified indophenol blue method (Mulvaney 1996), and nitrate concentration was determined using the vanadium (III) chloride method (Doane and Horwáth 2003) with a spectrophotometer (PD-303S; APEL, Japan). Inorganic N concentration was then calculated using equation (2):

Inorganic N = ammonium N + nitrate N ----------- (2)

#### 4.2.4 Statistical analysis

To examine whether PM application had a different effect on soil organic matter content and microbial activity in soils with, one-way analysis of variance (multiple comparisons) was performed followed by the least significant difference test. The correlation between the inorganic N content and increment in microbial biomass C was measured using Pearson's correlation method. All statistical analyses were performed using SPSS 19.0 with a significance level of $P < 0.05$.

## 4.3. Result

### 4.3.1 The total carbon, nitrogen content and microbial activity change for one year

As table 4-1 shows, at first, applied of 7.5% (v/v) of PM into soil (A, B and C) which contained high total carbon, application of PM did not promote the total carbon significantly. However, after 6 months, in treatment A, B, C and D application of 7.5% of PM did not increase total carbon content significantly ($p \leq 0.05$). After one year, application of 15% (v/v) PM into all soils can increase total carbon content significantly ($p \leq 0.05$). In soil (A to E), the decrease of total carbon in soil focused on first 6 months. And in soil F, the total carbon content decreased gradually. And In soil G, decrease of total carbon in soil focused on the later six months.

As table 4-2 shows, it can be found that, compare with the control group which is not application of PM, the decrease of total nitrogen for soil which is applied with PM is less than control group for each soil. At first, only in soil (E, F and G) application of 7.5% (v/v) PM promote total N content in soil. After 6 months from mixed with PM, application of 7.5% (v/v) PM promote the soil total N in soil D to G, increased the total nitrogen content significantly ($p \leq 0.05$). And after one year passed from mixed with PM into soils, application of 7.5% (v/v) PM promoted all soils. Application of 15 and 30% (v/v) of PM in all soils, promoted the total nitrogen content significantly.

As table 4-3 shows, at first after applied with PM into different soil, compare with the control group there is significant promotion on microbial activity ($p \leq 0.05$). After 6 months, in soil (C, D and E), there is no between 7.5% of application of PM into soil and control group. After one year applied with PM, in soil (C to G), there is no significant different among 7.5%, 15% of application of PM and control group, compare among three times of FDA activity through one year, the FDA activity decreased all the time, however the decrease of FDA in different soil (A to E) was focused on first 6 months, and in soil F the FDA of soil was decreased gradually, and in soil G the decrease was focus on later 6 months.

**Table 4-1. Carbon content in different treatments through one year**

| The harvest time | Different soil** | The total carbon content in different treatments | | | |
|---|---|---|---|---|---|
| | | 0* | 7.5%* | 15%* | 30%* |
| 2016.09.06 | A | 110.4 ± 9.01a | 116.1 ± 9.63a | 124.4 ± 10.66b | 142.2 ± 11.72b |
| | B | 76.0 ± 6.09a | 83.6 ± 6.34a | 92.7 ± 6.75b | 112.4 ± 8.69b |
| | C | 64.8 ± 5.34a | 68.9 ± 5.32a | 83.1 ± 6.27b | 96.5 ± 7.06c |
| | D | 43.3 ± 3.02a | 49.4 ± 4.03b | 61.4 ± 5.36c | 83.9 ± 7.29d |
| | E | 18.0 ± 0.98a | 24.4 ± 2.01b | 33.7 ± 1.45b | 52.4 ± 3.18c |
| | F | 11.9 ± 0.42a | 18.8 ± 0.72b | 26.4 ± 1.09c | 44.8± 2.39d |
| | G | 2.6 ± 0.21a | 7.9 ± 0.61b | 15.41 ± 0.82c | 32.1± 1.64d |
| 2017.03.06 | A | 94.7±8.91a | 100.7±11.22a | 111.1±12.35b | 123.3±9.54b |
| | B | 60.9±5.44a | 62.5±9.89a | 70.4±8.76b | 85.3±7.44b |
| | C | 52.8±8.31a | 54.2±3.43a | 60.3±4.31c | 70.3±6.43c |
| | D | 31.7±4.67a | 43.3±6.31a | 51.6±5.56c | 60.7±3.89d |
| | E | 9.8±2.11a | 12.2±1.33b | 14.2±3.23c | 29.2±5.31d |
| | F | 3.8±0.99a | 6.9±1.32b | 10.8±1.34c | 21.3±2.33d |
| | G | 1.7±0.34a | 6.5±1.90b | 8.9±2.31c | 18.8±1.58d |
| 2017.09.06 | A | 86.5±9.89a | 90.3±5.43a | 98.2±7.34a | 108.8±14.74b |
| | B | 53.7±5.44a | 54.2±4.19a | 55.2±7.21b | 75.4±7.55c |
| | C | 48.3±4.33a | 49.9±4.16a | 52.4±4.54c | 60.4±6.89c |
| | D | 28.5±2.34a | 29±3.13a | 41±3.13b | 52.8±5.54c |
| | E | 7.4±3.11a | 11.5±1.25b | 11.9±1.09b | 20.4±1.43c |
| | F | 2.1±1.02a | 5.1±0.98b | 8.3±2.12c | 16.2±2.78d |
| | G | 1.1±0.21a | 4.8±0.16b | 5.8±0.89b | 13.5±0.65c |

*** Percentage of compost made from pruning materials (PM) added to the soil.**

**** A: forest soil alone; B: 1:1 ratio of forest soil:subsoil; C: 1:2 ratio of forest soil:subsoil; D: subsoil alone; E: 1:1 ratio of subsoil:sand; F: 1:2 ratio of subsoil:sand; G: sand alone.Different lower-case letters within a column indicate significant differences among different concentration of application of PM into soils (*p* < 0.05).**

## Table 4-2. Nitrogen content in different treatments through one year

| The harvest time | Different treatment** | The total nitrogen of different individual plant | | | |
|---|---|---|---|---|---|
| | | 0* | 7.5%* | 15%* | 30%* |
| 2016.09.06 | A | 8.5 ± 0.73a | 8.7 ± 0.78a | 9 ± 0.87a | 9.6 ± 0.95b |
| | B | 5.7 ± 0.49a | 6.0 ± 0.51a | 6.3 ± 0.55a | 7.2 ± 0.7b |
| | C | 4.8 ± 0.43a | 5.0 ± 0.43a | 5.4 ± 0.51b | 6.0 ± 0.57b |
| | D | 3.1 ± 0.24a | 3.4 ± 0.32a | 3.9 ± 0.43b | 4.9 ± 0.59b |
| | E | 1.3 ± 0.08a | 1.6 ± 0.08b | 2.0 ± 0.11b | 2.9 ± 0.25c |
| | F | 0.8 ± 0.03a | 1.1 ± 0.05b | 1.5 ± 0.08b | 2.4 ± 0.19c |
| | G | 0.2 ± 0.01a | 0.4 ± 0.04b | 0.8 ± 0.14b | 1.6 ± 0.13c |
| 2017.03.06 | A | 7.5a ± 1.01a | 7.8 ± 1.11ab | 8.1a ± 1.22b | 8.3 ± 1.34b |
| | B | 3.9 ± 0.76a | 4.3 ± 0.84b | 5.6 ± 0.92b | 6.6 ± 1.01b |
| | C | 4.3 ± 0.45a | 4.7 ± 0.50ab | 5.4 ± 0.54b | 5.8 ± 0.59b |
| | D | 1.1 ± 0.330a | 3.6 ± 0.36b | 4.7 ± 0.39c | 4.9 ± 0.43c |
| | E | 0.7 ± 0.11a | 1.6 ± 0.12b | 1.9 ± 0.13b | 2.3 ± 0.14b |
| | F | 0.4 ± 0.23a | 1.5 ± 0.25b | 2.2 ± 0.28b | 2.7 ± 0.31b |
| | G | 0.11 ± 0.01a | 1.2 ± 0.22b | 1.4 ± 0.31b | 1.7 ± 0.15b |
| 2017.09.06 | A | 6.8 ± 0.81a | 7.6 ± 0.89b | 7.9 ± 0.98b | 8.1 ± 1.08c |
| | B | 3.4 ± 0.60a | 4.0 ± 0.67b | 5.1 ± 0.74b | 5.7 ± 0.80b |
| | C | 2.9 ± 0.36a | 3.9 ± 0.39b | 4.2 ± 0.44b | 4.6 ± 0.47b |
| | D | 0.9 ± 0.26a | 3.1 ± 0.29b | 3.7 ± 0.32b | 4.1 ± 0.35b |
| | E | 0.5 ± 0.09a | 1.5 ± 0.10b | 1.8 ± 0.11b | 1.9 ± 0.12b |
| | F | 0.3 ± 0.18a | 1.4 ± 0.20b | 2 ± 0.22c | 2.1 ± 0.24c |
| | G | 0.06 ± 0.01a | 0.8 ± 0.10b | 0.9 ± 0.22b | 1.3 ± 0.17b |

*** Percentage of compost made from pruning materials (PM) added to the soil.**

**** A: forest soil alone; B: 1:1 ratio of forest soil:subsoil; C: 1:2 ratio of forest soil:subsoil; D: subsoil alone; E: 1:1 ratio of subsoil:sand; F: 1:2 ratio of subsoil:sand; G: sand alone.Different lower-case letters within a column indicate significant differences among different concentration of application of PM into soils ($p < 0.05$).**

**Table 4-3. Microbial activity content in different treatments through one year**

| The harvest time | Different treatment** | The FDA activity in different treatments | | | |
|---|---|---|---|---|---|
| | | 0* | 7.5%* | 15%* | 30%* |
| 2016.09.06 | A | 1.20±0.44a | 1.59±0.056b | 1.58±0.055b | 1.70±0.069c |
| | B | 1.04±0.37a | 1.32±0.044b | 1.35±0.053c | 1.60±0.055d |
| | C | 0.69±0.18a | 0.96±0.036b | 1.08±0.044c | 1.14±0.048d |
| | D | 0.42±0.09a | 0.86±0.038b | 1.03±0.035c | 1.06±0.046c |
| | E | 0.29±0.01a | 0.38±0.012b | 0.47±0.018c | 0.58±0.022d |
| | F | 0.20±0.03a | 0.32±0.011b | 0.38±0.02c | 0.46±0.015c |
| | G | 0.19±0.01a | 0.24±0.07b | 0.27±0.04bc | 0.32±0.012c |
| 2017.03.06 | A | 0.59±0.034a | 0.61±0.056a | 0.64±0.072b | 0.70±0.087c |
| | B | 0.41±0.021a | 0.47±0.06a | 0.55±0.030b | 0.66±0.036c |
| | C | 0.33±0.056a | 0.37±0.061a | 0.45±0.080b | 0.60±0.096b |
| | D | 0.29±0.031a | 0.31±0.0341a | 0.38±0.044b | 0.57±0.053b |
| | E | 0.21±0.019a | 0.25±0.033a | 0.32±0.042b | 0.44±0.051b |
| | F | 0.13±0.009a | 0.19±0.017b | 0.26±0.022b | 0.31±0.026b |
| | G | 0.10±0.004a | 0.18±0.05b | 0.23±0.015b | 0.31±0.018b |
| 2017.09.06 | A | 0.36±0.022a | 0.51±0.024b | 0.55±0.031b | 0.61±0.037c |
| | B | 0.30±0.043a | 0.42±0.047b | 0.47±0.030bc | 0.50±0.023c |
| | C | 0.24±0.062a | 0.28±0.068a | 0.30±0.088a | 0.41±0.06b |
| | D | 0.20±0.039a | 0.22±0.042a | 0.22±0.053a | 0.43±0.066b |
| | E | 0.13±0.006a | 0.14±0.006a | 0.15±0.008a | 0.31±0.010b |
| | F | 0.08±0.011a | 0.10±0.012a | 0.12±0.015a | 0.25±0.018b |
| | G | 0.04±0.019a | 0.08±0.020a | 0.10±0.037a | 0.23±0.032b |

*** Percentage of compost made from pruning materials (PM) added to the soil.**

**** A: forest soil alone; B: 1:1 ratio of forest soil:subsoil; C: 1:2 ratio of forest soil:subsoil; D: subsoil alone; E: 1:1 ratio of subsoil:sand; F: 1:2 ratio of subsoil:sand; G: sand alone.Different lower-case letters within a column indicate significant differences among different concentration of application of PM into soils ($p < 0.05$).**

### 4.3.2 Change in microbial biomass C and inorganic N during early application stage

Details about the changes in microbial biomass C in the control and treatment groups are shown in Fig 4-1a. Microbial biomass C generally decreased in soils F and S after incubation. However, although a similar decreasing trend was observed after mixing the two soils with PM, microbial biomass C increased between days 7 and 22. The increment in microbial biomass C due to the application of PM in the two soil types is shown in Fig 4-1b, which clearly shows an increase followed by a decrease. The magnitude of the increment was larger in soil F than in soil S.

Details about the change in the inorganic N concentration in the control and treatment groups are shown in Fig 4-2A and 4-2B, and differences in terms of N concentration between the soils mixed with PM and those alone are shown in Fig 4-2C and 4-2D. When soil F was mixed with PM, the inorganic N concentration decreased until day 22 and then increased to reach a level that was greater than that of soil F alone by approximately day 30. When soil S was mixed with PM, the inorganic N concentration initially decreased until day 22 and then began increasing until it was greater than the concentration of soil S alone by approximately day 51. Correlation analysis revealed that the inorganic N concentrations in soils F and S mixed with PM were negatively correlated with the increment in microbial biomass C (Fig 4-3).

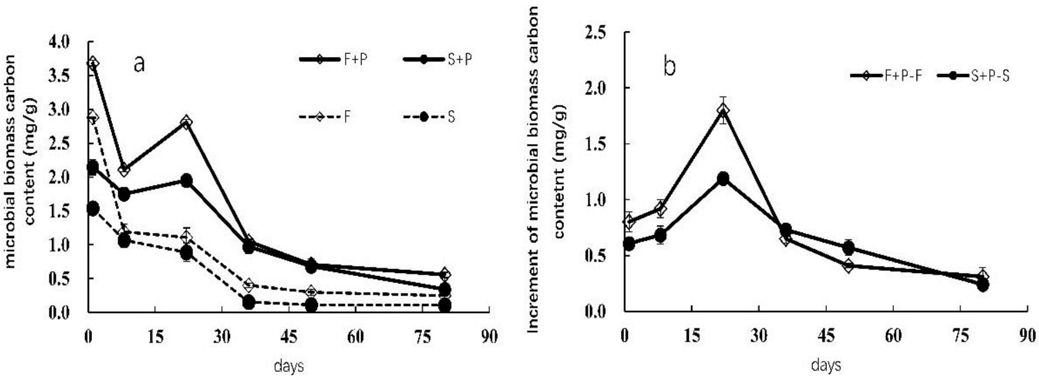


**Fig4-1 (a) The microbial biomass carbon change in different soils and soils mixed with pruning materials (PMs) compost. Fig2-1 (b) The increment of microbial biomass carbon content change in different soils mixed PM. F is forest soil (soil F) only, S is subsoil (soil S) only, F+P is soil F soil mixed with PM, S+P is soil S mixed with PM. F+P-F is increment of the microbial biomass carbon content in forest soil mixed with PM. S+P-S is increment of the microbial biomass carbon content in soil S mixed with PM**

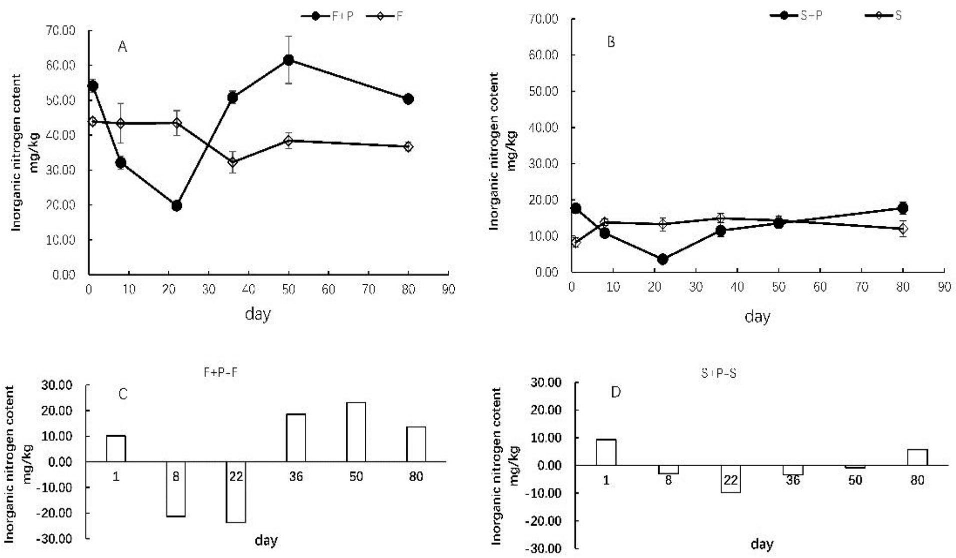


**Fig 4-2 The inorganic nitrogen ($NH_4^+$ and $NO_3^-$) concentration change in different control groups and treatments. (A) F is soil F only and F+P is soil F mixed with compost made from pruning materials (PMs), (B) S is soil S only, S+P is soil S mixed with PM. (C) Difference of inorganic nitrogen concentration between soil F and soil F with application of PM. (D) Difference of inorganic nitrogen concentration between soil S and soil S with application of PM.**

## 4.4. Discussion

### 4.4.1 The effect of application of PM on soil C, N and soil microbial activity for one year

There is about 400g $kg^{-1}$ total C content in PM (Table 3-1), therefore, at first application of 15% and 30% (v/v) PM into all soils, increased the soil total carbon content obviously in all soils. Comparing with C content, the total N is relative smaller. Therefore, because of the relative smaller content of total N, there is obviously influence on soil total N content with only 30% (v/v) of PM applied into soil with high total C and N content.

PM contains numerous of microorganisms (López-González et al. 2015) and a large amount of mineralizable C, which is a resource for soil microorganisms (Liu and Takahashi 2019). Consequently, the application of PM to the different soil types significantly increased their microbial activity.

In soil with high microbial activity, such as forest soil and soil mixed with subsoil, the C mineralization is faster than in soil with low microbial activity. Therefore, in soil with high microbial activity, after 6 months, the effect of PM application on increasing soil total C is not obviously. However, because of application of PM into soil increased the soil microbial biomass such as actinomycetes (Takahashi et al. 2015), and the capacity of fixation nitrogen is positive correlated with some soil actinomycetes (Franche et al. 2009), therefore over time application of PM lead to improved total N content in all soils compared with control group. Because of maintain soil microbial activity is depend on soil mineralizable carbon content (Table 4-4), in soil with high

**Table 4-4. The correlation among change of total C, N and microbial activity in soil with 0, 7.5% (v/v), 15% (v/v) and 30 % (v/v) PM application.**

| Amount of PM application (0%) | microbial activity |
|---|---|
| total N | 0.29 |
| total C | 0.91** |
| Amount of PM application (7.5 %) | microbial activity |
| total N | 0.46 |
| total C | 0.82** |
| Amount of PM application (15 %) | microbial activity |
| total N | 0.16 |
| total C | 0.80** |
| Amount of PM application (30%) | microbial activity |
| total N | 0.17 |
| total C | 0.85** |

*** reflect the significant correlation at p< 0.05**

**** reflect the significant correlation at p< 0.01**

microbial activity, from 6 months later, the reduce of microbial activity is more obviously. The correlation among reduce amount of total C, N and microbial activity was shown in Table 4-4, the reduce amount of microbial activity is positive correlated with the reduce amount of total C, and there is no significant correlation between reduce amount of total N and microbial. Therefore, it can be found that the mineralizable C is the necessary to maintain soil microbial activity.

### 4.4.2 The dynamic of microbial biomass and N

The application of PM to soils caused the amount of mineralizable C to decrease due to organic matter mineralization, which generally resulted in decreased microbial biomass C. However, it increased from days 7 to 22 (Fig 4-1a); this suggested that PM had a priming effect on the soil samples, causing some of the stable C content to be converted into mineralizable C that can be used by microorganisms (Chen et al. 2014). Therefore, the increase in microbial biomass C is correlated not only with mineralizable C content of PM but also with the C content of soil (Blagodatskaya et al. 2014).

The increment in microbial biomass C rapidly increased following the application of PM to the soils (Fig 4-1b), whereas inorganic N content rapidly decreased (Fig 4-2) due to multiplying microorganisms requiring large amounts of inorganic N and thereby causing N immobilization (He et al. 2000; Zimmerman et al. 2011). The negative correlation observed between inorganic N concentration and increment in microbial biomass C in soils F and S mixed with PM (Fig 4-3) indicates that inorganic N is affected by the increment in microbial biomass C that occurs following the application of PM. Because soil F had a higher original microbial activity than soil S, it exhibited a faster decrease in increment in microbial biomass C. Consequently, N mineralization was probably faster in soil F than in soil S following the application of PM and the period of N immobilization was probably shorter (Henriksen and Breland 1999; Deng et al. 2016). Supporting this, we found that the period of immobilization of inorganic N was approximately 25 days for soil F and 45 days for soil S following the application of PM (Fig 4-2C and D). Thus, the period of N immobilization is shorter when PM is added to soils with high microbial activities.

The application of PM to forest soil and subsoil caused the amount of mineralizable C to decrease due to organic matter mineralization, which generally resulted in decreased microbial biomass C. However, it increased from days 7 to 22 (Fig 4-1a); this suggested that PM had a priming effect on the soil samples, causing some of the stable C content to be converted into mineralizable C that can be used by microorganisms (Chen et al. 2014). Therefore, the increase in microbial biomass C is correlated not only with mineralizable C content of PM but also with the C content of soil (Blagodatskaya et al. 2014). The increment in microbial biomass C rapidly increased following the application of PM to the soils (Fig 4-1b), whereas inorganic N content rapidly decreased (Fig 4-2A) due to multiplying microorganisms requiring large amounts of inorganic N and thereby causing N immobilization (He et al. 2000; Zimmerman et al. 2011). The negative correlation observed between inorganic N concentration and increment in microbial biomass C in soils F and S mixed with PM (Fig 4-3) indicates that inorganic N is affected by the increment in microbial biomass

C that occurs following the application of PM. Because soil F had a higher original microbial activity than soil S, it exhibited a faster decrease in increment in microbial biomass C. Consequently, N mineralization was probably faster in soil F than in soil S following the application of PM and the period of N immobilization was probably shorter (Henriksen and Breland 1999; Deng et al. 2016). Supporting this, we found that the period of immobilization of inorganic N was approximately 25 days for soil F and 45 days for soil S following the application of PM (Fig 4-2 C and D). Thus, the period of N immobilization is shorter when PM is added to soils with high microbial activities.

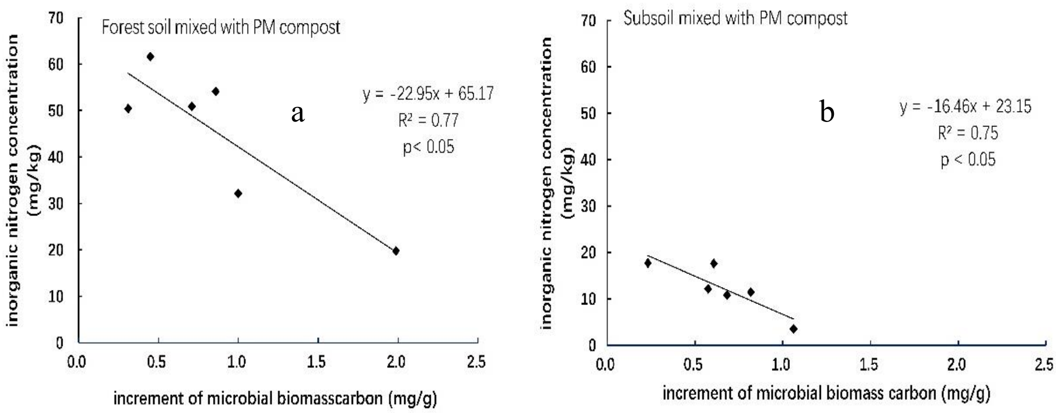


**Fig 4-3. The relationship between increment of microbial biomass carbon content due to application of PM and inorganic nitrogen concentration in soil mixed with PM. (a) is in soil F (b) is in soil S.**

## 4.5. Conclusion

Compared with small amount of PM application, sufficient PM application into soils with different organic matter content and microbial activity, increased soil total C, N content and microbial activity obviously, indicated that different amount of PM application lead to different effect on soil organic matter content obviously contributed to improve soil biological properties and organic matter content. Application of same amount of PM into soil, the duration of maintaining soil microbial activity is negative correlation with soil original microbial activity. In soil with high microbial activity, once a year application of PM into soil, over 30% (v/v) is necessary use for maintaining soil microbial activity. In same soil, larger amount of PM application promotes soil organic matter content and microbial activity for longer time. The phase of N starvation caused by PM application is negative correlated with the original soil microbial activity.

# Chapter 5 General discussion and Conclusion

## 5.1 The characteristics of PM

### 5.1.1 The original characteristics of PM

Comparing with conventional compost (dung, leaves and bark compost) There is higher mineralizable C content in PM than conventional composts (Liu et al. 2019). Because of the texture characteristics of PM, comparing with conventional compost, the mineralization process of PM in soil is longer. And the necessary energy for PM mineralization is similar with the other plant composts (Liu et al. 2019), therefore PM can be use in same environment with other conventional plant compost.

In addition, comparing with dung compost, there is smaller content of inorganic N content (Atiyeh et al. 2001; Benito et al.2005), PM showed adequate levels of organic matter and correspondingly high cation-exchange capacity (CEC) values (Liu et al. 2016). The C/N ratio varied between 22 and 48, significantly higher than the optimal values of 15–20 (Benito et al. 2005).

### 5.2.2 The effect of PM application on soil properties

Sufficient of PM promotes the soil total C and N content obviously in all kinds of soils (chapter 3). Because of the high microbial biomass and mineralizable content in PM (López-González et al. 2015), application of PM into soil promoted the soil microbial activity effectively (chapter 3). Therefore, PM can be used to amend the soil in wildness, desertization area, post-disaster reconstruction and so on.

Comparing with conventional compost, the effect of PM on soil improving soil pH, total C, N and CEC is almost same with conventional composts (Liu et al. 2016).

However, comparing with conventional compost, application of pruning materials compost (PM) into soil, supply more mineralizable C as resource to microorganisms thereby maintain microbial activity in soil for longer time (section 4.1, chapter 2). And because of the larger amount of mineralizable C content, application of PM into soil caused the priming effect on soil organic matter and organic matter of PM (chapter 3). Therefore, application of PM into soil caused microorganism multiplying N immobilization during early stage after mixed with PM into soil (He et al. 2000). And because of the lack of inorganic N content in PM (Atiyeh et al. 2001; Benito et al.2005), there is N starvation appeared with PM application in soil (chapter 3).

Meanwhile comparing with mineralization characteristics of PM mixed in forest soil and subsoil, mineralize rate of PM in forest soil is faster than in subsoil. Therefore, the phase of PM mineralization in soil with high microbial activity is shorter (chapter 3, section 3.2). The turnover of N in PM after mixed with soil is correlated with the PM mineralizing (Hobbie 2015), therefore, the phase of N immobilization caused by application of PM into soil is different in soils with different microbial activity (chapter 3, section 3.2). However, because of the different phase of C mineralization with PM which is applied into soils with different soil microbial activity (Hobbie 2015), the decrement of total C, N content and soil microbial activity in soils with different organic matter content and microbial activity for same time (chapter 3, section 3.1). The effect duration of PM on promoting soil organic content and microbial activity is different (chapter 3, section 3.1).

Due to the fast reduce of C content in soil with high microbial activity, reduce of microbial activity in soil with high original microbial activity is faster than in soil with low microbial activity for every 6 months (chapter 3, section 3.1). Therefore, PM application into soils with different soil microbial activity is different, application PM into soil with low soil microbial activity, the effect is more obviously than in soil with high soil microbial activity. Meanwhile the effect duration of PM on promoting soil organic matter content and microbial activity is longer in soil with low organic matter content and microbial activity than in soil with high organic matter content and microbial activity. And with more amount of PM application, the effect on promoting soil organic matter content and microbial activity is more obviously and longer.

### 5.2.3 The effect of PM application on plant growth

The beneficial effects of compost on plant growth and soil quality are reported in literature such as application of compost improve soil biological diversity contributed to inhibit the plant disease, supply the available nutrients to plants and improve the soil organic matter content and microbial activity and water holding capacity (Hoitink et al. 1997; Atiyeh et al. 2001). And the effect caused by compost are directly related to the physical, chemical and biological properties of the composts (He et al., 1995).

Comparing with dung compost, Application of PM into soils with different organic matter content and microbial activity caused rapidly microorganisms multiplying (section 3.2, chapter 3), because of the N immobilization caused by rapidly microorganisms multiplying (He et al. 2000), there is N starvation for plant growth with no capacity of N fixation (section 3.1, chapter 4). Therefore, except for planting plants with capacity of N fixation, planting plants in surface of soil with low microbial activity which is applied with PM due to the long phase of N starvation for plants, there is inhibition occurred on plant growth, and the more amount of PM application caused inhibition easier (chapter 4, section 3.1 and 3.2). However, in soil with high microbial activity such as forest soil, the process of N immobilization is relative shorter (chapter 3, section 3.2), therefore feedback of PM is easier to achieve, so that available nutrients are supplied to plants by PM feedback, therefore appropriate amount of PM application into soil with high microbial activity such as forest soil, promote plant growth immediately. Through comparing with planting plants with capacity of N fixation and without capacity of N fixation, there is no inhibition occurred on plant growth with capacity of N fixation (chapter 4, 3.2 section), therefore it can be concluded that, except for N starvation, there is no other negative effect on plant growth.

Through planting Komatsuna (*Brassica rapa* var. *perviridis*) three times for one year concluded that Over 6 months after mixed with PM, the microbial activity and total C content decreased obviously (chapter 3, section 3.1), and the N starvation is correlated with microbial biomass increasing, thus because of the obviously reduce of total C and microbial activity, the N starvation and inhibition on plant growth disappeared with 7.5% (v/v) of PM applied into all soils. And over one year, inhibition of 15% and 30% (v/v) PM application on plant growth disappeared in all soils except the soil with extreme condition such as sand (chapter 4, section 3.1).

## 5.2 The practical use of PM

Because of the litter management in urban, there is lack of organic matter content appearing in soil in urban park and other green space (Takahashi et al. 2000). In addition of urban green space, there are many other places are need amend with compost. Such as somewhere suffered nature disasters, desertization area and son. Therefore, the application of compost for soil restoration in sandy field, wildness or someplace which suffered nature disasters occurred are necessary to be considered.

Sufficient of PM promotes the soil total C and N content obviously in all kinds of soils (chapter 3). Because of the high microbial biomass and mineralizable content in PM (López-González et al. 2015), application of PM into soil promoted the soil microbial activity effectively (chapter 3). Therefore, PM can be used to amend the soil in urban park, wildness, desertization area, post-disaster reconstruction and so on. In addition, in agriculture, continue application of chemical fertilizers also caused the lack of organic matter content. Therefore, the PM also can be used as amendments for soil in agriculture.

Because of the larger amount of mineralizable C content in PM than conventional compost (Liu et al. 2009), and there is higher C/N ratio in PM (Benito et al. 2009). Therefore, the PM application lead to the inorganic N immobilization in soil after mixing with PM. Although PM application improves the soil chemical properties such as (CEC, base saturation and acidity) (Liu et al. 2016) and physical properties such as soil hardness and water holding capacity and soil aeration (Takahashi et al. 2009), in soil with low microbial activity there is a longer N starvation in order to inhibit plant growth.

In conclusion, the advantage of PM for amending soil is inexpensive (Zhang et al.2013) and lasting supply resource to microorganisms in soil for longer time (Liu et al.2019). Meanwhile the disadvantage is due to the large mineralizable content and low content of inorganic N, PM application lead to N starvation in soil (chapter 3) in order to inhibit plant growth (chapter 4).

### 5.2.1 PM application in soil with high organic matter content and microbial activity in agriculture or in some green space with deep root vegetations.

Applying PM into soil with high microbial activity such as forest, grassland and so on soils, because of the high soil microbial activity lead to high rate of organic matter mineralization, Sufficient PM application also can maintain the microbial activity for lone. Thus, in cause of improving soil properties which contains high microbial activity, in order to decrease the application frequency lead to reduce the construction cost the sufficient PM is necessary. Because of the process is short until PM achieve feedback of available nutrients to soils, application of PM improves soil organic matter content and microbial activity mean while promote plant growth. Therefore, applying PM into soil with high microbial activity the application method can be conducted as Fig 5-1 shows, applying large of PM into soil, before the organic matter content and microbial activity come back to the original state, start the second application and Reciprocate.

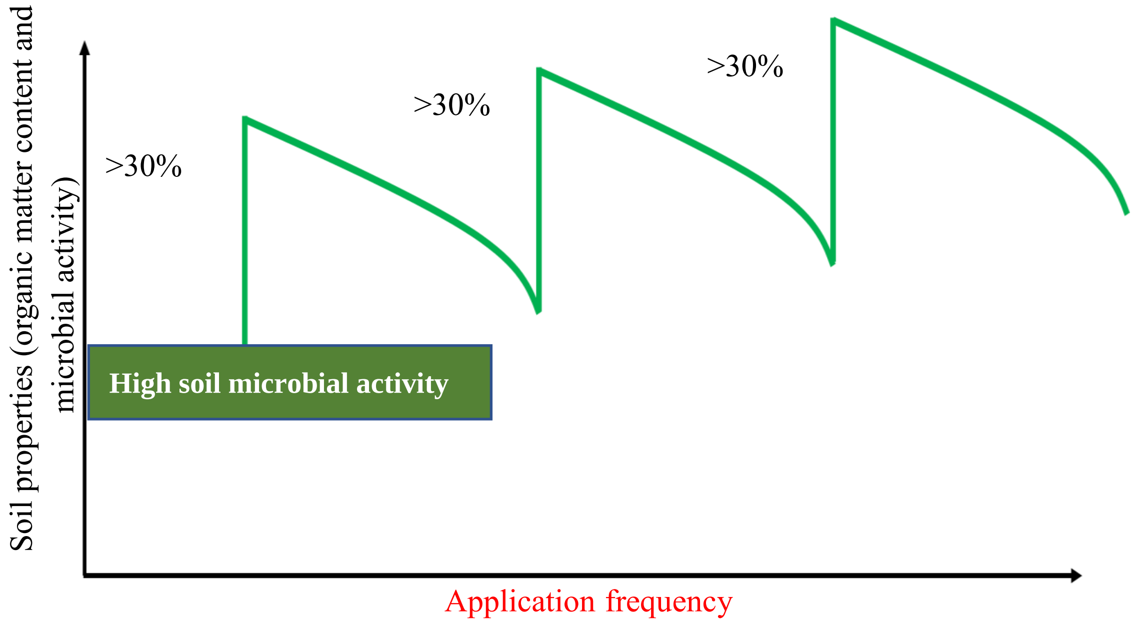


**Fig 5-1 The appropriate application method of PM in agriculture soil with high microbial activity of agriculture or in green space with deep root vegetations.**

### 5.2.2 PM application in soil with low organic matter content and microbial activity in agriculture or green space with shallow rooted vegetations.

Because of the larger mineralizable C content in PM, N starvation in soil caused by PM application. The phase of N starvation is negative correlated with soil microbial activity (chapter 4), therefore in soil with low microbial activity, PM application inhibit plant growth without capacity of N fixation due to the long phase of N starvation caused by PM application. Meanwhile more PM application causes the inhibition on plant growth more obviously due to the larger mineralizable C caused the longer phase of N starvation.

Therefore, in soil with low microbial activity, PM application caused the N immobilization in soil, however the low microbial activity lead to longer phase of N immobilization contributed to inhibit the N absorb by plants because of the N starvation. In order to inhibit plant growth.

**To improve the soil properties in soil with low microbial activity meanwhile avoid the inhabiting on plants, the appropriate methods were proposed as follow:**

**1)Application of PM with different application amount and frequency**

In soil with low microbial activity and applying PM itself without addition materials, because of the low mineralize capacity in soil, the turnover of PM in soil is slowly, therefore the N starvation occurred easily, thus the application method of PM into soil with low organic matter content and microbial activity as Fig 5-2 shows. At first utilize relative smaller amount of PM into soil with high frequency, then increase of the application amount gradually contributed to improves soil properties such as microbial activity gradually, when the soil microbial activity arrived at high enough, the application method of PM can be same with the application method used in soil with high microbial activity as Fig 5-1 with low application frequency. This operation can improve soil organic matter content and microbial activity in soil with low organic matter content and microbial activity meanwhile try to avoid inhibiting on plant growth.

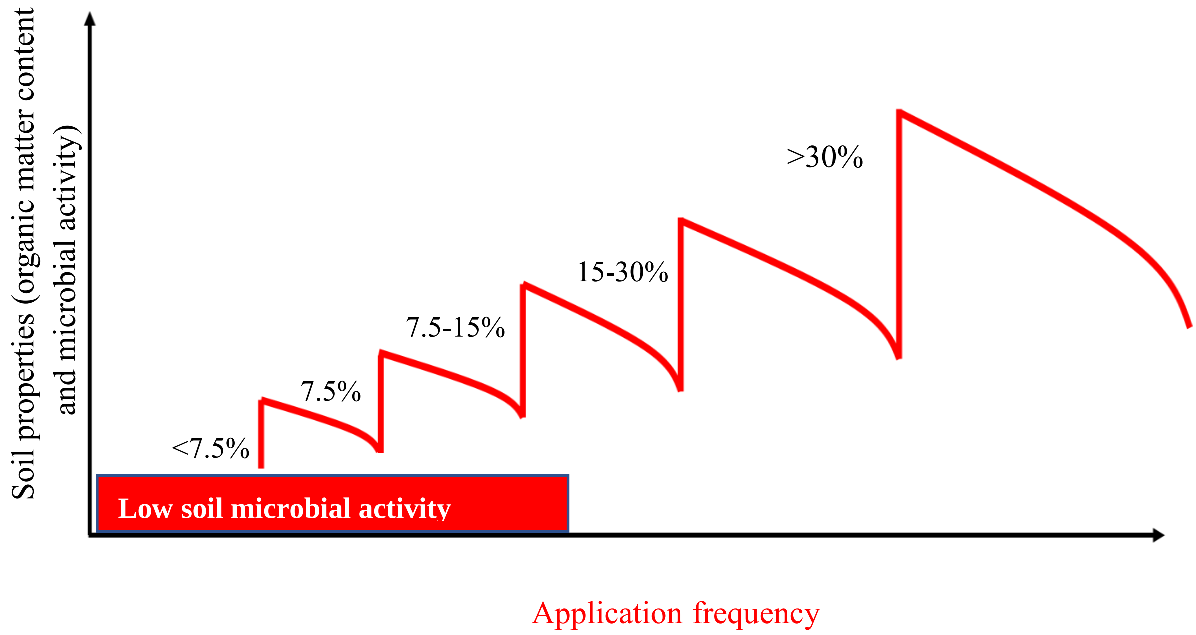


**Fig 5-2 The appropriate application method of PM in soil with low microbial activity of agriculture or in green space with shallow rooted vegetations.**

**2) Application of PM with addition N fertilizes or high N content materials together**

Yamamoto et al. 2011 reported that such as in Machida city in Tokyo, because of the lack of available nutrients in PM, the PM is almost used with cooperation materials, such as moth, dung, oil pot and so on. In addition of mixing with cooperation materials, special treating method for PM in agriculture is also be selected to make the utmost of PM in agriculture.

Therefore, at some situation with application of PM, The PM can be used with N fertilizers or compost with high inorganic N content such as oil pot, dung compost together, therefore the N starvation in soil can be ignored. Therefore, the application method can be selected as Fig 5-3. To decrease the application frequency, at beginning, the PM can be applied with sufficient amount of PM and N fertilizers or high N content compost together.

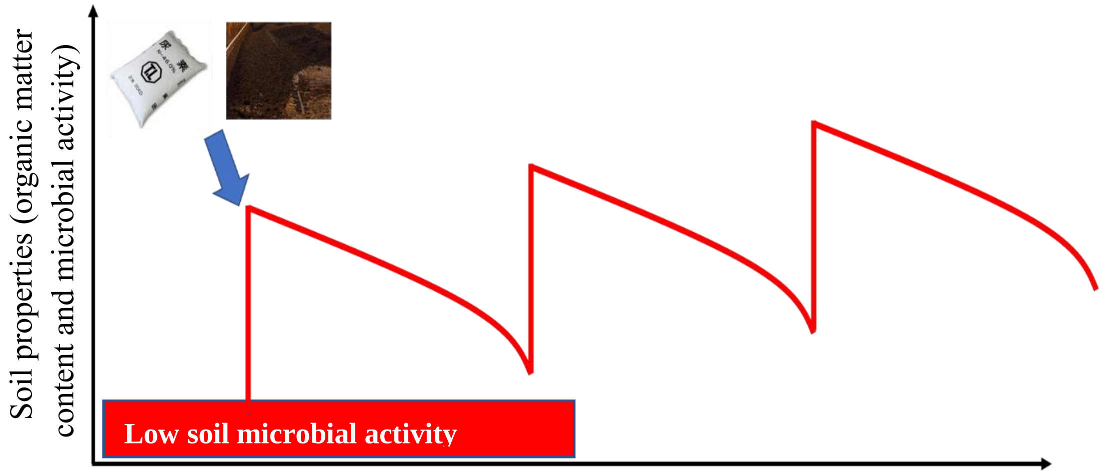


**Fig 5-3 The appropriate application method of PM in soil with low microbial activity of agriculture or in green space with shallow rooted vegetations (application with supplement materials).**

**3) Setting a mature period of PM in soil before planting**

As we know from chapter 4, after 6 months from applied PM into soil, there is no inhibiting on plant growth in all soils with 7.5% and 15% in volume PM application. And after one year from applied PM into soil, there is no inhibiting on plant growth in all soils with 30% in volume PM application. Therefore, in agriculture and setting a free period between applying PM and planting can improve soil properties with application of PM meanwhile avoiding the inhibiting on plant growth. With PM application, the soil microbial activity increasing, the mature period for PM in soil is becoming shorter and shorter (Fig 5-4).

In addition, Yamamoto et al. 2011 reported that before applying with PM into soil, take a secondary fermentation in special place, then applying into soil, will also avoid the inhibiting effect on plant growth.

Therefore, setting a mature period for PM before planting is another method to improve soil properties meanwhile avoid the inhibition caused by PM application on plant growth.

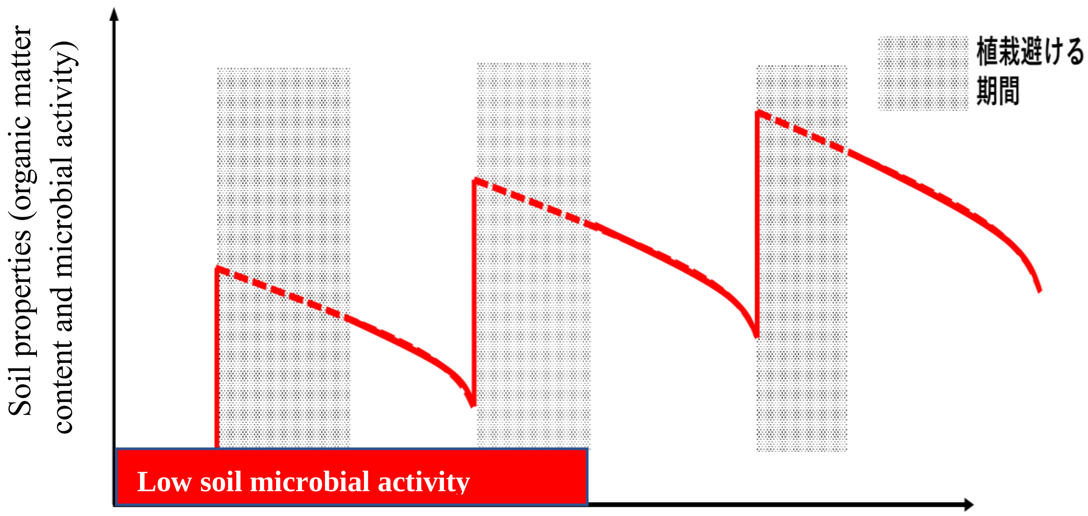


**Fig 5-4 The appropriate application method of PM in soil with low microbial activity of agriculture or in green space with shallow rooted vegetations (setting mature period).**

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