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Soil Microbial Biomass and Diversity Amended with Bagasse Mulch in Tillage and No-tillage Practices in the Sugarcane Plantation

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Abstract

Biomass residues in plantation farms and process industries still have valuable materials and can be recycled as other materials. Gunung Madu Plantation (GMP) in Lampung Province, Indonesia where this study was observed had used bagasse as mulch 80 tons (wet weight) per hectare. This study observed the effect of tillage and bagasse much on soil microbes in sugarcane plantation. Each treatment was used in conjunction with or without bagasse mulch in a split-plot experimental design. Previous study showed bagasse mulch had increased litter fungal biomass and communities in the soil. However soil microbial community structure has not been comprehensively investigated. Quinone profile method was used to analyze the community structure amended with bagasse mulch in the sugarcane plantation. Quinone profile method reflectively estimated the microbial biomass and the diversity. The no-tillage with bagasse mulch had the highest microbial biomass (1.026 μmol kg^{-1} dry soil) compare to the no-tillage without bagasse mulch, the conventional tillage with bagasse mulch and the conventional tillage without bagasse mulch. The Diversity index (DQ) and Shannon-Wiener diversity index (H) was also the highest in the no-tillage amended with bagasse mulch. Therefore mulch treatment in combination with no-tillage is an effective residue management of biomass residue to improve soil quality.

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Nomenclature	
NT	No-tillage without bagasse mulch
NTM	No-tillage with bagasse mulch
CT	Conventional tillage without bagasse mulch
CTM	Conventional tillage with bagasse mulch
UQ	Ubiquinone
MK	Menaquinone
DQ	Diversity based on quinone profile
H'	Shanon's diversity index

1. Introduction

1.1. Overview

Intensive cultivation in sugarcane plantation could impact to crop productivities, nutrients in the soil and soil biota. Meanwhile, processing biomass produces commercial products and also biomass residues. Biomass residue such as bagasse is a valuable material that can be used as organic mulch.

There are many kinds of activities that could improve soil conditions such as tillage, fertilization and mulching. In this research, conventional tillage (a tillage system that used cultivation as major means), organic mulch (bagasse mulch), organic and synthetic fertilizer were used. All activities should be managed, not only for plant production but also for environmental sustainability.

Previous study by Miura [1] at the same study site showed that bagasse mulch had increased litter fungal biomass and richness. Meanwhile, these study focuses on soil microbial biomass and diversity amended with bagasse as organic mulch in tillage and no-tillage practices in the sugarcane plantation based on Quinone profile

Soil quality is influenced by microbial process. Thus the relationship among the activity, size, and composition of microbial community in soil should be elucidated. However sensitive methods have been lacking for determining temporal changes in the activity, size, and composition of the soil microbial communities [2]. The quinone profile method has been successful to elucidate the changes in microbial community structure in soil with different fertilization history [2,3]. The total amount of quinone has a linier relationship to microbial biomass in soil [4]

1.2. Objective

The objective of this study was to evaluate the microbial biomass and diversity in soil using Quinone profiling method, in the sugarcane plantation area, under factorial design of tillage (conventional, and no-tillage) and mulching with bagasse as an organic residue.

2. Materials and Methods

2.1. Site description

The sugarcane (*Saccharum officinarum*) plantation area (4º40'S, 105º13'E, altitude c.a 45 m) is in Sumatra, Indonesia. The site was in a large plantation area (approximately 25,000 ha). Split plot design was used with soil tillage as the main factor and bagasse mulch as the secondary factor. Each plot was 25 x 25 m and 5 m buffer zone from the road. Furthermore, 80 tons (wet weight) per hectare of organic BBA fertilizer (five parts of bagasse, three parts of blotong of filter cake, and three parts bagasse ash) were spread before plough in the conventional tillage plots. Meanwhile, 80 tons of organic BBA fertilizers were spread after planting in the no-tillage plots. Inorganic fertilizers (N; P; K 120:80:180 kg/ha) were spread in all treatments at the time of planting.

The plantation area was treated with tillage and no-tillage combine with bagasse mulch and without bagasse mulch to observe microbial community structure. In the conventional tillage practice plots were ploughed three times to dept of 20 cm (first), 40 cm (second), and 20 cm (third) in July 2010. For the plots that amended with bagasse mulch treatment, 80 tons (wet weight) per hectare of bagasse mulch were spread on the soil in August 1 until August 5, 2010.

2.2 Soil sampling

Soil samplings were collected first at initial condition (before treatment), in June 2010, and then at time of harvesting in July 2011. Four treatments were used in this research: the conventional tillage with bagasse mulch (CTM), the conventional tillage without bagasse mulch (CT), the no-tillage with bagasse mulch (NTM) and the no-tillage without bagasse mulch (NT). There were four treatments each with five replications.

2.3. Qunone profile method

Studies on microbial community structure have become increasingly important in terms of soil functions. For this research, method that was being used was quinone-profiling method. The quinone profile analysis was utilized as an indicator and useful tool to characterize the microbial community structure in sewage, sediment and also soil [5-7]

Microbial quinone in soil analysed is based on the procedure as previous studies [5, 6]. Microbial quinone was extracted from 20 g soil sample with a mixture of chloroform and methanol (2:1, v/v) filtered using Whatman no.2 filter paper. Then, crude quinone was purified by extraction with hexane and acetone, purification and separation of quinones with Sep-Pak. The Sep-Pak® Plus Silica (Waters Co., Tokyo).

Hexane, 5 ml, was passed through the Sep-Pak cartridges, and then the hexane extract of quinones was loaded onto the cartridges at a fixed flow rate of 24 ml/min. Then menaquinone was eluted with 20 ml of 2% diethyl ether in hexane solution and ubiquinone was eluted with 20 ml of 10% diethyl ether in hexane solution. The elutes of ubiquinone and menaquinone from the Sep-Pak (or each fraction of the elutes) were evaporated to dryness, and then the evaporated residues were re-dissolved in acetone for future quantitative analysis by HPLC.

The extracted microbial quinone was analysed using high performance liquid chromatography, HPLC (Shimadzu, Japan). In this study, the types and concentrations of quinones were determined with HPLC equipped with an ODS column (Zorbax-ODS, 4.6 (I.D.) × 250 mm, Shimadzu-Dupont) and a multi-channel UV detector (photo diode array detector, model: SPD-M10A, Shimadzu). A mixture of methanol and di-isopropyl ether (9:2, v/v) was used as the mobile phase at a flow rate of 1.0 $ml \cdot min^{-1}$. The temperature of the column oven was maintained at 35°C.

Quinone components can be separated by HPLC with a mixture of methanol-isopropyl ether, methanol-isopropanol, or methanol-chlorobutane as the mobile phase [8-10]. Quinone species were identified according to the retention time on the column and the UV spectrum of each peak observed in the multi-channel UV detector. The use of a photodiode array detector facilitates the identification of quinone species by recording their absorption spectra [11]. In a methanol-isopropyl ether mixture (9:2, v/v), menaquinone show absorption maximal at 270 nm and ubiquinone at 275 nm.

The linier relationship between the logarithm of the retention times of quinones and the equivalent number of isoprenoid unit (ENIU) was also used to identify the quinone type [12, 13]. Ubiquinone with 10 isoprenoid was used as the quantitative standards for ubiquinone and menaquinone, respectively. Ubiquinone 10 can be purchased from a manufacture. Unfortunately, most menaquinone species and other quinone derivatives found in nature are not commercially available. When the logarithmically transformed HPLC retention times of quinone isoprenologs are plotted against the number of isoprene units, a linier relationship is found for each quinone series. As many microorganisms contain one major isoprenoid quinone, the diversity of quinone species indicates the diversity of microbial group containing the quinone species. This is another advantage in the use of quinone profiles for characterization of a microbial community. Classical concepts of diversity involve richness of species (number of species) and equitability (or evenness) in the distribution of populations. Diversity indices are functions of the number of species of a given taxonomic group existing in an ecosystem, the number of individuals or fraction of each species, and the form of population distribution. The greater the number of species and the higher the evenness in the population or fraction of each species, the larger the values of diversity indices become [14]. For

characterization by quinone profiles, a diversity index for quinone profiles (DQ) has been proposed by Hu [13].

$$DQ = \left(\sum_{k=1}^{n} \left(\sqrt{f_k} \right) \right)^2 \quad (1)$$

where, f_k is the mole fraction of the quinone component, and n denotes the number of quinone species.

Shanon's diversity index *(H')* was also calculated by the equation;

$$H' = -\sum_{k=1}^{n} f_k \ln f_k \quad (2)$$

The microbial equitability was calculated by the equation;

$$\mathrm{EQ} = \frac{\mathrm{DQ}}{\mathrm{n}} \quad (3)$$

When the fractional contents of all quinine species are equal each other EQ becomes equal to 1. On the other hand, when a dominant quinone species takes a larger fractional content than other species EQ value becomes decrease.

2.4. Statistical analysis

The soil microbial biomass was analyzed by using split plot two-way analysis of variance (ANOVA). Multiple comparison analysis and t-test were used for comparing Qunone profile in each treatment. The statistical analysis was performed using R 2.7.2 [17].

3. RESULTS AND DISCUSSION

3.1. Microbial biomass

The Figure 1 shows the total microbial biomass before and after treatments. The microbial biomass based on quinone content before treatment was 0.265µmol kg^{-1}dry soil and then after NT treatment, the quinine content was increased to 0.578 µmol kg^{-1}dry soil. The quinone content of the NTM treatment reached a peak, 1.026 µmol kg^{-1} dry soil. Similarly, the CTM treatment grew around 0.417 µmol kg^{-1} dry soil. In contrast, quinone content in the CT treatment was slightly decreased which became 0.232 µmol kg^{-1}dry soil.

The experimental results indicated that microbial biomass were abundant in the NTM treatment as shown in Figure 1. It might be because the no-tillage treatment had fewer disturbing activities to the soil than the tillage treatment. In addition, in the no-tillage treatment, weed biomass was high. Previous study [1] reported that the high weed biomass in the no-tillage increased fungi and litter moisture. Wardle [18] reported that weed residue contains higher available nutrients than crop residues for microbial biomass and increase soil respiration. Therefore might be not only bagasse as crop residues gave contribution to microbial biomass but also the weed. Furthermore bagasse mulch as crop residue mulch and fertilizers contribute to soil microbes.

Thus, it was observed that there was considerable increase in total biomass in the no-tillage without bagasse mulch, the no-tillage with bagasse mulch, and the tillage with bagasse mulch. Statistical analysis showed that there were significant differences in each treatment. Furthermore, it was observed that the soils treated with NT, NTM and CTM each showed significant difference when compared with that of the 'before treatment'. Therefore no-tillage and bagasse mulch had relation to increase microbial biomass.

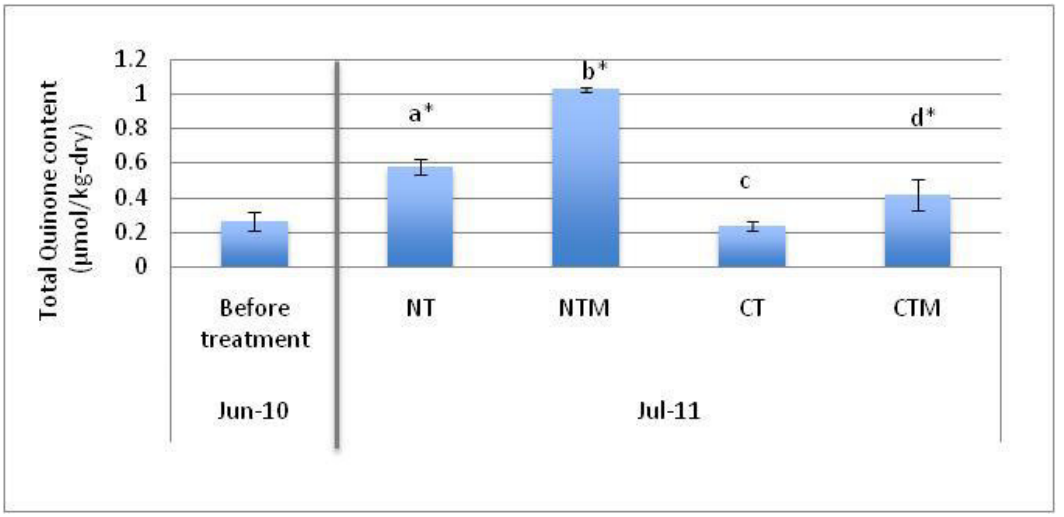

Fig 1. Total microbial biomass based on quinine content. No significant difference in the column name starting with the same alphabet or asterisk at $p > 0.05$ by Tukey HSD.

Quinone components can be separated as two major parts; ubiquinone (UQ) and menaquinone (MK). According to Hirashi [15], respiratory quinone (including ubiquinone and menaquinone) exist in the bacteria gaining energy by way of respiration. Ubiquinones are used for aerobic or nitrate respiration. Meanwhile, menaquinone is for aerobic or anaerobic respiration.

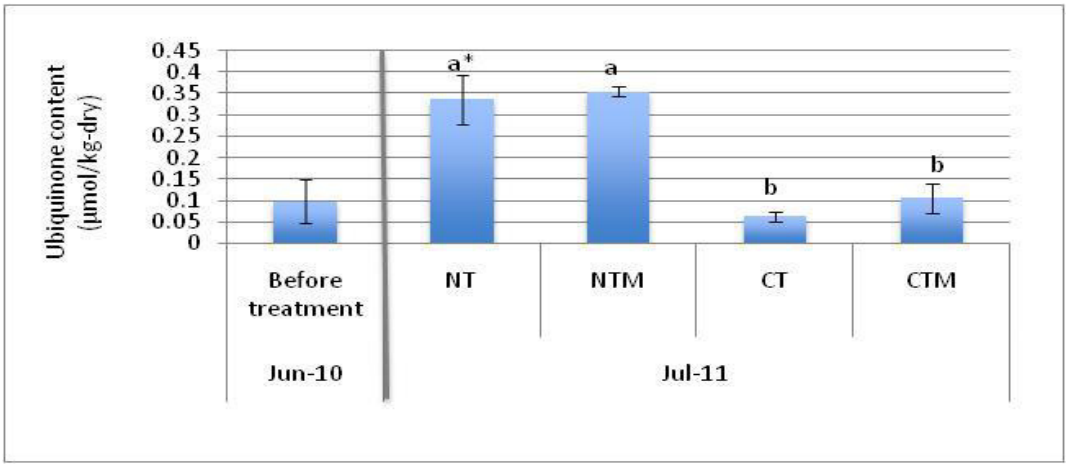

Fig 2. Total amount of ubiquinone (UQ). No significant difference in the column name starting with the same alphabet or asterisk at $p > 0.05$ by Tukey HSD.

Figure 2 shows the value of the UQ biomass for the NT treatment was 0.336µmol kg^{1}dry soil and the NTM treatment was 0.355 µmol kg^{-1}dry soil. There was not much difference in the values of UQ between the NT treatment and the NTM treatment. The UQ biomass for the CT treatment was 0.062 µmol kg^{-1}dry soil and the CTM treatment was 0.105 µmol kg^{-1}dry soil. There was also no significant difference between the CT treatment and the CTM treatment.

Meanwhile, there was a major difference in UQ biomass for the no-tillage (NT and NTM) and the tillage (CT and CTM) treatments. It seems that no-tillage treatment gave significant contribution to the UQ biomass. In

addition, the UQ biomass between the before treatment and NT treatment were significantly different.

Figure 3 shows the menaquinone (MK) biomass in each condition. The MK biomass for the NT treatment was 0.242 µmol kg^{-1}dry soil, the NTM treatment was 0.67 µmol kg^{-1}dry soil, the CT treatment was 0.181µmol kg^{-1}dry soil, and the CTM treatment was 0.312µmol kg^{-1}dry soil.

The MK biomass at the CTM treatment and the CT treatment were considerably different. The MK biomass for the NT treatment and NTM treatment were also significantly different. This suggests that bagasse mulch made significant difference for MK biomass. Moreover, the MK biomass for the NTM treatment was significantly different with all treatments in July 2011. In addition, the MK biomass between the before treatment soil and the no-tillage (NT and NTM) and CTM treatments also showed significant difference.

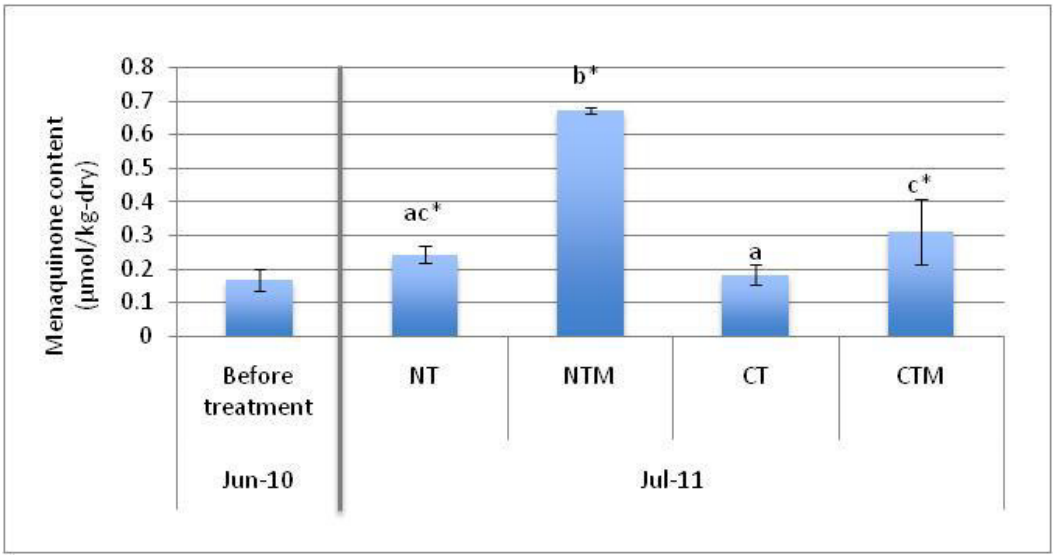

Fig 3. Total amount of Menaquinone (MK). No significant difference in the column name starting with the same alphabet or asterisk at $p > 0.05$ by Tukey HSD.

The Figure 4 shows the values of ratio UQ/MK before treatment was 0.634, then it decreased to 0.376 after the CTM treatment, decreased to 0.529 after the NTM treatment and slightly decreased to 0.391 after the CT treatment. This suggests that aerobic bacteria in the soil before the treatment might be higher than aerobic bacteria in the CT, CTM and NTM. Soil was mixed for tillage treatments and it might cause disturbance to the aerobic bacteria around surface area. Meanwhile organic mulch was covering the surface soil. The soil samplings were collected 0 – 5 cm from the surface. On the other hand, the value of UQ/MK for the NT treatment increased to 1.415.

Previous study about analysis of the difference in microbial community structure [19] showed the UQ/MK ratio for suspended microorganisms which is higher than sessile microorganism. It suggest that the ratio of aerobic bacteria in suspended bacteria might be higher than that in riverbed microbial film.

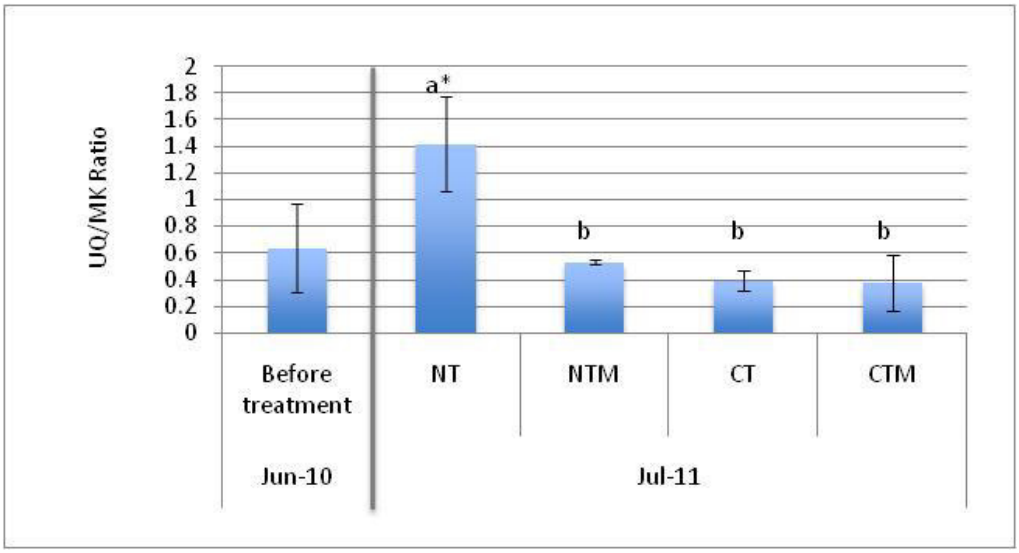

Fig 4. The ratio values of UQ/MK. No significant difference in the column name starting with the same alphabet or asterisk at $p > 0.05$ by Tukey HSD.

3.2. Microbial diversity and equitability

The values of diversity index based on Hu [13] and Shanon's diversity index (*H'*) are shown in Table 1. The values of diversity index and Shanon's diversity index of the quinine were slightly similar to that of the NT, CT and the CTM treatment. The highest diversity occurred for the NTM treatment, because no-tillage combined with bagasse mulch provided better condition for soil and microbes to grow. Mulch treatment is effective in the prevention of surface soil erosion. Crop residue mulch and fertilizers are recommended for sustaining soil quality [16].

Table 1. Values of diversity based on quinone compositions

Soil	*H'*	*DQ*	*EQ*
NT	2.77±0.08	21.47±0.72	0.631±0.02
NTM	2.89±0.02	23.48±0.39	0.675±0.01
CT	2.75±0.10	20.97±1.76	0.680±0.03
CTM	2.73±0.08	20.50±1.33	0.669±0,02

The "±" values denote the range of diversity in five replications

To describe the equitability of the distribution of quinone species, an index of equitability of quinone species (EQ=DQ/n) was developed based on the concept of evenness index E (=*H'/H max*) [13, 14]. The microbial equitability (EQ) for total quinone in this study was in the order as follows the conventional tillage without mulch > the no-tillage with mulch > the conventional tillage with mulch > the no-tillage without mulch treatments.

4. Conclusion

This study relates to conventional tillage, and no-tillage practices which are amended with bagasse mulch in order to determine biomass and diversity of soil microbes. The no-tillage soil with bagasse mulch had the highest microbial biomass compared to all other treatments which includes no-tillage without bagasse mulch, the conventional tillage with bagasse mulch and the conventional tillage without bagasse mulch. The Diversity index (DQ) and Shannon-Wiener diversity index (H) were also the highest in the no-tillage amended with bagasse mulch. Therefore mulch treatment in combination with no-tillage is an effective residue management of biomass residue to improve soil quality.

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