Application of oil palm empty fruit bunch effects on soil biota and functions: a case study in Sumatra, Indonesia

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ABSTRACT

Oil palm (*Elaeis guineensis*) is an important tropical crop which provides one-fifth of the world's vegetable oil, yet its rapid expansion can negatively influence the soil ecosystem. Identifying suitable agronomic management such as crop residue application is important for the sustainable development of oil palm. We examined the effects of adding empty fruit bunches (EFB), a major oil palm residue, on multiple soil abiotic properties, soil biota, and indicators of soil functions. We compared treatments of EFB applications with three application rates, and a chemical fertilizer treatment in a 15–year trial in Central Sumatra, Indonesia. EFB application increased pH and aggregate stability in 0–10 cm soils and decreased the soil bulk density. EFB application increased the abundance of soil detritivore mites, soil fauna feeding activity, and soil microbial activity. EFB application decreased the biomass of a dominant invasive earthworm species, *Pontoscolex corethrurus* (Müller, 1857). Results from structural equation modelling suggested that EFB directly affected soil biota and functions, rather than through altering soil abiotic properties. The effects of EFB application on most soil abiotic properties, soil biota and function indicators were independent of the application rate. Our results revealed that EFB application has a high potential to enhance soil biota and functions in oil palm plantations.

1. INTRODUCTION

Soil provides crucial functions and ecosystem services to enhance food security, land restoration, and climate change mitigation, which are key Sustainable Development Goals of United Nations (S. D. Keesstra et al., 2016). Various interacting soil organisms and their processes contribute to key soil ecosystem functions, such as nutrient cycling, carbon sequestration, and soil structure maintenance (Bardgett and van der Putten, 2014). In agricultural ecosystems, management practices may influence abiotic soil environment and soil biota, which then alter key soil ecosystem functions and crop production (Birkhofer et al., 2008).

Identifying sustainable management is especially important for tropical production systems which produce 1.7 tons of dry crop yield annually (West et al., 2010). Oil palm (*Elaeis guineensis*) is a wide spread economic crop in Southeast Asia which produces one-fifth of vegetable oil globally (Kurnia et al., 2016; Sayer et al., 2012). The global oil palm land area covers 16.4 million ha, equivalent to ten percent of the world's permanent croplands (FAO, 2015). Land conversion from forest to oil palm can reduce soil biodiversity, increase nutrient leaching, and negatively influence soil carbon storage and fertility (Dislich et al., 2017). To identify and implement suitable soil management for mitigating these negative environmental impacts is important; however, few studies have examined these management practices and their effects on soil biodiversity and ecosystem functions in oil palm plantations(Bessou et al., 2017; Carron et al., 2015; Tao et al., 2016).

In most oil palm plantations in Southeast Asia, chemical fertilizer is used as a major source of nutrients. More recently, crop residues from oil palm have been used as organic fertilizers and mulch substrates to reduce the quantity of mineral fertilizers needed. One of the main oil palm residues from the palm oil extraction process are the empty fruit bunches

(EFB), which are the structural part of the fruit bunches (Chang, 2014). Several studies have reported the effects of EFB application on soil fertility and yield in Indonesian and Malaysian oil palm (Comte et al., 2013; Abolfath Moradi et al., 2014; Tao et al., 2017). However, there is little information on the effects of EFB application on soil biota and ecosystem functions (Carron et al., 2015; Tao et al., 2016), and whether the effects are direct or through altering soil abiotic properties. It is also unknown whether the effects are quantity-dependent, i.e., whether the magnitude of the effects depends on the application rate of EFB. For other crops such as apricot, olive, and persimmon, applying crop residue mulch has shown to reduce soil degradation (S. Keesstra et al., 2016; Parras-Alcántara et al., 2016; Ritsema, 2016). Relevant information for oil palm is crucial for plantation managers and policymakers to promote and implement suitable soil management practices to achieve sustainable intensification of oil palm.

Among soil organisms, earthworms can be litter transformers and soil engineers which structure the environment of other soil organisms, whereas soil mites directly feed on crop residues and microorganisms (Coleman et al., 2004). Crop residue application may influence soil microbial respiration and soil faunal feeding activities either by providing food resources, or by altering soil abiotic properties and microhabitats for soil biota; yet such mechanisms have rarely been explored in tropical agroecosystems especially for perennial crops (Kurzatkowski et al., 2004; Römbke et al., 2006). Moreover, the majority of research has focused on the effects of crop residue application on the litter layer rather than the soil mineral layer, despite the fact that carbon sequestration and nutrient cycling also happen in the soil layer (Ashford et al., 2013).

This study aimed to examine the long-term effects of oil palm residue addition on soil biota and functions in the top soil layer. Specifically, we asked: 1) does EFB application influence key soil biota and function indicators? 2) does EFB application directly influence soil biota and functions, or indirectly through altering soil abiotic properties? and 3) do the effects of EFB application on soil biota and functions depend on the application rate? We examined six soil abiotic properties, two soil biota groups, and two indicators of soil functions in a 15-year field trial in an oil palm plantation in Sumatra, Indonesia. We used a structural equation modelling approach (Grace et al., 2012; Shipley, 2013) to examine the potential causal relationships between soil abiotic properties, soil biota and functions. We hypothesized that EFB application may influence soil biota and functions both by directly providing resources, and by modifying soil abiotic properties. The soil biota could also have cascading effects on other soil fauna groups and functions. In addition, we expected that the responses of soil biota and functions to EFB application would be stronger at higher application rates.

2. MATERIALS AND METHODS

2.1 Site description and experimental design

The study was carried out in an oil palm plantation in Sumatra, Indonesia (0° 56’0” N 101°18’0”E). The oil palm plantation was established in 1987 and has been certified by the Roundtable on Sustainable Palm Oil (RSPO). The previous land use of this area was secondary tropical forest dominated by *Dipterocarp* species. The climate of this region is described as tropical humid, with a mean temperature of 26.8 °C and average rainfall of 2400 mm year^{-1}. The soils are Inceptisols (Typic Dystrudepts), within the loamy lowland soil class (USDA soil classification system).

The 15-year trial began in 1998, when the age of oil palms was 11 years. Field sampling and measurements were conducted at the end of the trial during September-October 2014. The field trial was established at two adjacent oil palm commercial plots. The field trial covered a total area of 36 hectares (1200 m length × 300 m wide) and was composed of five replicate blocks. Each replicate block was composed of four treatment plots, resulting in a total of 20 treatment plots (5 replicate blocks × 4 treatment plots). The replication number was five in a nested design (See **Supplementary S1**).

Each treatment plot (80 m × 40 m) was composed of 36 palm trees, arranged as eight palm trees in four rows. The applications were applied throughout the treatment plot continuously for 15 years. Soil sampling was undertaken at the end of 15 years at five oil palms in the central two rows. There was at least one palm between two focal palms to avoid spill over effects (**Supplementary S1**). Each treatment plot was surrounded by 1.5 m depth ditches to prevent interference by adjacent treatment plots.

The four treatment plots in each replicate block were: Low-EFB treatment applied with EFB (210 kg tree^{-1} yr^{-1}. equivalent to 30 t ha^{-1} yr^{-1}) and urea (0.02 kg tree^{-1} yr^{-1}); Medium-EFB treatment applied with EFB (420 kg tree^{-1} yr^{-1}, equivalent to 60 t ha^{-1} yr^{-1}) and urea (0.04 kg tree^{-1} yr^{-1}); High-EFB treatment applied with EFB (630 kg tree^{-1} yr^{-1}, equivalent to 90 t ha^{-1} yr^{-1}) and urea (0.06 kg tree^{-1} yr^{-1}), and chemical fertilizer treatment applied with a full quantity of chemical fertilizers without adding EFB. EFB as the structural part of fruit bunches after fruit removal contains dry weight of 45-49 % C, 0.5-1.0 % N, 0.02-0.05 % P, 1.3-2.2 % K, and 0.2-0.4 % Mg (Budianta et al., 2010; Chang, 2014; Kavitha et al., 2013; A. Moradi et al., 2014; Pardon et al., 2016). Chemical fertilizer treatment included the application of 1.75 kg tree^{-1} yr^{-1} urea (46 % N), 0.5 kg tree^{-1} yr^{-1} triple super phosphate (45 % P_2O_5, 15% Ca), 2.5 kg tree^{-1} yr^{-1} muriate of potash (61% K_2O, 46% Cl), and 0.05 kg tree^{-1} yr^{-1} Kieserite (16% Mg, S:22%). For detailed description of each treatment see **Supplementary S2**.

In EFB treatment plots, EFB was applied once a year for 15 years at one side of the harvesting paths, followed by urea application on the top of EFB to regulate the EFB decomposition rate. The application rate of the Low-EFB treatment was similar to standard operations in the oil palm industry, whereas Medium-EFB and High-EFB treatments represented alternative application rates of EFB. In the chemical fertilizer treatment, the type and quantity of chemical fertilizers were similar to conventional standard estate practices, and the fertilizers were applied within palm circles (1-2 m radius) twice a year (i.e. during the February-March and September-October periods) throughout the trial period. Harvesting path and palm circle account for approximately 5-15 % and 10-15% of the total plantation area, respectively, as the majority of oil palm plantations in Southeast Asia (Nelson et al., 2013).

2.2 Field measurements

We measured ten indicators for soil abiotic properties (soil pH, soil moisture, aggregate stability, bulk density, soil organic carbon and total nitrogen), soil biota (earthworm biomass and soil mite density), and soil ecosystem functions (soil fauna feeding activity and soil microbial activity). We examined these soil properties at the soil mineral layer rather than litter layer, since the response of soil-dwelling fauna communities and their processes under soil management practices are relatively unknown in the oil palm ecosystem (Tao et al., 2016). We examined these soil properties beneath EFB at one side of harvesting paths of EFB treatment plots, and at the equivalent positions in chemical fertilizer plots.

2.2.1 Measuring soil abiotic properties

We measured soil moisture using the WET sensor (Delta-T Device, UK). For soil pH, soil organic carbon, soil total nitrogen, and soil aggregate stability, one soil sample was collected at the depth of 0-15 cm under each focal palm. The resulting five soil samples for each treatment plot were bulked to form a composite sample. Samples were air-dried for 4-7 days and sieved through 2 mm and 0.5 mm for soil chemical analysis. Soil organic carbon concentration was measured using the Walkley-Black method (Nelson and Sommers, 1982). Total nitrogen was determined by the Kjeldahl method (Bremmer and Mulvaney, 1982). Soil pH was determined using a pH meter with a soil to water ratio of 1:1.

We analysed soil aggregate stability using the wet sieving method (Eijkelkamp, the Netherlands). Soils particles under 0.25 mm were used for the measurement. Unstable aggregates were extracted by physical shaking for fixed amounts of time, while stable aggregate was extracted by sodium hydroxide. The aggregate stability was calculated as the percentage of stable aggregates weight over total aggregate weight. For bulk density measurement, three soil samples of fixed volume were collected using metal rings (5 cm diameter and 5.1 cm depth) under each focal palm. The dry weight of the samples was measured after over-dry for 105 degrees for 24 hr. The bulk density was calculated as the weight per unit volume of a soil sample.

2.2.2 Measuring soil biota

Earthworms and soil mites were chosen as the focal soil biota groups in our study as they contribute to key functions in nutrient cycling (Coleman et al., 2004), and their abundance in the mineral layer of the soil. We used a modified Tropical Soil Biology and Fertility (TSBF) approach to extract earthworms (Moreira et al., 2008). Under each focal palm, the organic layer on the top-soil was removed by a shovel, and a soil monolith of 50 cm length × 20 cm width × 10 cm depth was excavated. Earthworms were hand-sorted by a team of five people in the field. The fresh weight of each earthworm was measured, stored in a

90% ethanol solution, and the taxonomy was identified by an expert (Dr Samuel Wooster James, Maharishi University of Management, United States). The biomass of earthworms (g m^{-2}) was obtained by dividing the fresh weights of earthworms by the surface area of the soil monolith.

We sampled soil mite using a modified Berlese-Tullgren funnel approach (Moreira et al., 2008). Three soil samples were collected using soil cores (7.5 cm diameter and 4.2 cm length) at each focal tree and pooled for the extraction over a 48-hour period. We identified soil mites into taxonomic groups at the suborder level (Krantz and Walter, 2009). We also collected other microarthropod groups i.e. Collembola and Formicidae from the extraction, yet the number was low and was not used for further analysis. The density of each Suborder (individuals/m^2) of each treatment plot was obtained by dividing the number of mites of each Suborder by the surface area of three soil cores.

2.2.3 Measuring indicators of soil functions

Soil fauna feeding activity represents direct litter feeding rates of soil mesofauna and macrofauna, therefore is an indicator for nutrient turnover in the soil. We measured soil fauna feeding activity by the bait lamina method (Terra Protecta GmbH, Berlin, Germany) (Von Torne 1990). The method uses thin PVC sticks (1 x 6 x 120 mm) with 16 apertures of 1.5 mm diameter and 5 mm apart, filled with standardized bait made of cellulose powder, bran flakes and active carbon in a ratio of 70:27:3. Under each of the focal palm, we placed six bait lamina sticks 40 cm apart in a row. The sticks were inserted vertically until the top aperture was just below the soil surface, and the bottom aperture was at a depth of 8 cm below the soil surface. The sticks were left for 3 days and bait consumption was recorded by assessing each aperture, i.e. feeding activity was recorded as 0 (without perforation = no evidence of feeding) or 1 (partial or complete perforation = evidence of feeding).

Soil microbial activity is the metabolic process of micro-organisms which associated with oxidizing soil organic carbon and nitrogen-containing compounds. In this study, we examined the potential of heterotrophic respiration under *ex-situ* conditions, by measuring CO_2 burst after moistening dry soil for 24hr (Solvita CO_2-Burst Test, USA, https://solvita.com/co2-burst/). This method is different from usual measurements of *in-situ* basal soil respiration in field conditions which examine real-time soil respiration.. Rather, it measures potential of microbial respiration by water induction as used in various ecological studies (Muñoz-Rojas et al., 2016a, 2016b; Ward et al., 2017). To perform the test, each composite soil sample used for chemical property analysis was air-dried and sieved through a 2mm mesh sieve. Subsequently, 40g of each sample was placed in a 50ml plastic beaker and then inside a 240ml incubation jar. The soils were moistened by adding 20ml of water into the jar. A CO_2 probe was placed inside the jar before incubation at room temperature for 24hr. After incubation, the CO_2 probe was taken out from the jar and measured using a spectrometer (digital color reader). The background of atmospheric CO_2 was taken into account for the reading..

2.3 STATISTICAL ANALYSIS

All statistical analyses were performed in R.3.3.1 (R Core Team, 2016). We used linear mixed effects models to examine the effects of EFB application on soil abiotic properties, soil biota, and soil ecosystem functions. The treatment type (Low-EFB, Medium-EFB, High-EFB, and chemical fertilizer treatment) was fitted as a fixed effect (categorical variable). Treatment plots nested within replicate blocks were fitted as random effects. To correct for heterogeneity in the residuals, earthworm biomass and soil mite density were log (x+1)-transformed, bulk density and soil fauna feeding activity were square- root transformed, and the rest of the variables were log-transformed. For soil mite, both the total soil mite density and the Suborder of Oribatida were tested against the treatment type. Significant overall effects of treatment type were further explored using a post-hoc Tukey test. All the statistical tests were evaluated using $\alpha = 5\%$. Mixed effects modelling was performed using the *lme* function of the *nlme* package (Bates et al., 2015), and the Tukey test using the *multcomp* package (Hothorn et al., 2008).

Structural equation modelling was performed to test whether the EFB application influenced soil biota and the soil ecosystem functions by direct provision of food resources, or via modifying soil abiotic properties. This approach allows for evaluating pre-existing theories using observational data, with a focus on exploring possible mediation pathways (Eisenhauer et al., 2015; Grace et al., 2012). Among the ten indicators, soil aggregate stability was not included in the structural equation modelling as it positively correlated with soil bulk density. We thus used only soil bulk density to represent soil structure. In addition, neither soil organic carbon nor total nitrogen were included in the structural equation modelling, because they did not show significant responses to the treatment type from prior data exploration. For the remaining seven indicators (soil pH, soil moisture, bulk density, earthworm biomass, mite density, soil fauna feeding activity, and soil microbial activity) an *a prior* model was built with potential causal relations between variables based on theoretical knowledge and hypotheses (**Supplementary S3**).

Subsequently, we built a component model for each of the seven indicators. The component model was in a form of a mixed effect model, with fixed effects based on hypothesized causal pathways in the *a prior* model, and random effects as treatment plots nested within replicate blocks. The response variables were log (x+1)-transformed or square-root transformed when necessary. The normal distribution and homogeneity of variances of the residuals of each component model of the global path model were graphically checked. From prior data exploration, the responses of most indicators to EFB treatments were not dependent on the application rate of EFB. We thus assigned the treatment type as a categorical variable with two levels (one level as the Low-EFB, Medium-EFB, and High-EFB treatments, and the other level as the chemical fertilizer treatment). The unbalanced sample size of the two levels was dealt with by mixed effects models (Pinheiro and Bates, 2000). A structural equation model including the seven component models was further simplified using a stepwise approach to remove non-significant relationships (**Supplementary S4**). We tested the effects of these removals on the Akaike Information Critetion (AIC), AIC with a correction for finite sample sizes (AICc), and the model fit. During the model selection process, new causal relationships between the soil variables which were not included in the *a prior* model appeared, and were further included for examination. The most parsimonious model was selected where deleting any variables generated ΔAICc < 3 (Shipley, 2013) (**Supplementary S5**). The model goodness-of-fit was examined by the Shipley's test of d-separation, using Fisher's C statistics with X^2 distribution (Lefcheck, 2016). The structural equation modelling was performed using the *piecewiseSEM* package (Lefcheck, 2016).

3. RESULTS

3.1 EFB application effects on soil abiotic properties

After 15 years of continuous treatment, soil pH, soil moisture, aggregate stability, and bulk density at 0-15 cm soil depth significantly differed among treatments (**Figure 1, Supplementary S6**). Soil pHand aggregate stability were significantly higher under EFB treatments of all application rates, compared to the chemical fertilizer treatment. The positive effects of EFB treatment on soil moisture increased with the application rate, whereas this quantity-dependent response was not observed for soil pH and aggregate stability. Bulk density was significantly lower under EFB treatments of all application rates than in the chemical fertilizer treatment. Soil organic carbon and total nitrogen levels did not significantly differ among the four treatments, yet this could be due to high variations in the data.

3.2 EFB application effects on soil biota and functions

A total of 168 earthworms were collected, with a mean density of 1344 ind m^{-2}. The total earthworm biomass under the Medium-EFB and High-EFB treatments was significantly lower compared to the chemical fertilizer treatment, while the biomass was similar between Low-EFB treatment and the chemical fertilizer treatment (**Figure 2, Supplementary S6**). Across all the treatment plots, the dominant earthworm species was the invasive endogeic species *Pontoscolex corethrurus* (Müller, 1857). No epigeic or anecic earthworms were found in our study sites.

A total of 1168 mites were extracted, with a mean density of 909 ind m^{-2}. The total mite density under the chemical fertilizer treatment was significantly lower than Low-EFB and High-EFB treatments (**Figure 2, Supplementary S6**). The total mite density was similar between Medium-EFB treatment and the chemical fertilizer treatment. The extracted mites belonged to four main suborders: Astigmata, Oribatida, Mesostigmata, and Prostigmata. Across the treatments, the dominant groups were Astigmata and Oribatida, with their abundance accounted for 68% and 22% of all the specimens, respectively. The density of Oribatida under the Low-EFB treatment was significantly higher than the chemical fertilizer treatment and Medium-EFB treatment. The dominant group of Astigmata in our samples was *Astigmata hypopus* (Supercohort Desmonomatides, Orbatida).

The soil fauna feeding activity under the Low-EFB and Medium-EFB treatments was significantly higher than the chemical fertilizer treatment (**Figure 2, Supplementary S6**). The High-EFB treatment had similar levels of soil fauna feeding activity as the chemical fertilizer treatment. Soil microbial activity under all rates of EFB treatments was higher than chemical fertilizer treatment (**Figure 2, Supplementary S6**).

3.3 Direct v.s. indirect effects of EFB application

We used structural equation modelling (SEM) to examine whether EFB treatment affected soil biota and functions by providing resources, or through altering soil abiotic properties. Compared to chemical fertilizer treatment, EFB treatment increased soil pH (path coefficient=0.35, P<0.001) and soil moisture (path coefficient=0.19, P<0.05), and decreased soil bulk density (path coefficient=–0.37, P<0.001) (**Figure 3**). EFB application directly decreased the total biomass of *Pontoscolex corethrurus* (path coefficient=–0.23, P<0.05). EFB application directly increased the abundance of detritivore mites (path coefficient=0.25, P<0.01) and soil fauna feeding activity (path coefficient=0.19, P<0.01), rather than indirectly through modifying soil abiotic properties. EFB application increased soil microbial activity both by providing resources (path coefficient=0.26, P<0.001), and through increasing the soil fauna feeding activity (path coefficient=0.18, P<0.05) (**Figure 3, Supplementary S5**). Linear mixed effects models confirmed that soil fauna feeding activity positively influenced soil microbial activity ($F_{1,70}$=8.75, P=0.0042) (**Figure 4**).

4. DISCUSSION

This study examined the effects of EFB application on soil abiotic properties, soil biota, and ecosystem functions in a 15-year trial in Central Sumatra, Indonesia. We used structural equation modelling to understand whether the effects of EFB application directly influenced soil biota and ecosystem functions, or indirectly through altering soil abiotic properties. We synthesized whether the effects of EFB application on these soil ecosystem indicators depended on the application rate of EFB.

4.1 EFB application effects on soil abiotic properties

EFB application over 15 years significantly increased soil pH (**Figure 1**), confirming the capacity of EFB in releasing base cations to alleviate soil acidification and improve the nutrient retention of the soil (Bakar et al., 2011; Comte et al., 2013). EFB application also enhanced soil aggregate stability and decreased bulk density, suggesting its positive effects on soil physical properties and structure (Abolfath Moradi et al., 2014). EFB application did not markedly increase soil organic carbon levels at our study site. However, the high variations in the data may cause the absence of statistic differences. Uneven distribution of EFB at sampling palms and potential differences in microclimate between sampling palms may have led to variations in EFB decomposition. Soil organic carbon appeared to decline with higher rates of EFB treatment (**Figure 1**), suggesting that more inputs of urea with higher rate of EFB treatments increased the decomposition of existing soil organic carbon.

4.2 EFB application effects on soil biota and functions

The total earthworm biomass was significantly reduced under EFB treatments of all application rates, compared to the chemical fertilizer treatment. EFB contains high content of lignin (285 g kg^{-1}) and releases a phenolic compound of 2,6- bis (1,1-dimethylethyl) during field decomposition (A. Moradi et al., 2014; Sabrina et al., 2012). Some phenol compounds are known to be toxic for earthworms (Sabrina et al., 2012) and thus may be a cause of earthworm decline under EFB treatment in our study, yet further study is needed to confirm this hypothesis. In addition, EFB mulched on the soil surface may create an anoxic environment which inhibits earthworm populations (Carron et al., 2015). The dominant earthworm population across all treatments was the invasive endogeic species, *Pontoscolex corethrurus*. This species is known to act as a “compacting species” under environments with scarce soil organic carbon, For example, it is reported to produce large and compact casts, which result in increased soil bulk density and macro-aggregates in the soil of some disturbed ecosystems (Alegre et al., 1996; Chauvel et al., 1999; Hallaire et al., 2000). In contrast, the structural equation modelling did not show significant impacts of earthworms on soil bulk density in our study. It is therefore likely that the small population size of *P. corethrurus* in our study site had limited effects on soil structure, and that EFB addition may directly decrease soil bulk density by protecting the soil from the compacting effect of the heavy tropical rain.

The density of detritivorous mites of Astigmata and Oribatida were higher under the EFB application compared to the chemical fertilizer treatment. The positive effects of crop residue management on soil mites were also found in other cropping systems (Minor and Norton, 2004; Sánchez-Moreno et al., 2009; Scheunemann et al., 2015). The effects of organic matter addition on soil detritivorous mitea can be due to the provision of trophic or energy sources, or by altering soil physico-chemcial environment (Bedano et al., 2006; Coleman et al., 2004). Soil animals such as endogeic earthworms may also have top-down control on soil mite populations through resource competition, direct feeding, and microhabitat disturbance (Eisenhauer, 2010; Mueller et al., 2016). Results from our structural equation modelling suggested that the soil mite density was strongly influenced by the bottom-up control of the EFB application, rather than top-down control by endogeic earthworms.

Prostigmata, an important predatory mite taxa, was absent under the chemical fertilizer treatment, while a few individuals were found under the EFB treatments. The appearance of Prostigmata under EFB application suggests increased regulatory functions of the soil food webs that are essential for energy and organic matter turnover (Wissuwa et al., 2012).

EFB application enhanced soil fauna feeding activity, coincided with another study examining soil heterogeneity between different management zones of oil palm (Tao et al., 2016). Soil macrofauna and mesofauna, such as earthworms, collembola, and soil mites have been reported to be the major feeders on the bait lamina assay in temperate regions and laboratory manipulations (Helling et al., 1998; Kratz, 1998). Our results suggested that EFB application directly enhanced the soil fauna feeding activity, rather than indirectly altering soil abiotic conditions or soil mite and earthworm populations. This result suggested that the EFB may serve as a food/energy resources for soil biota groups other than soil mites and earthworms (i.e. termites). The enhanced soil fauna feeding activity under EFB application also implies a more rapid turnover of carbon and nutrient cycling with the addition of EFB, which can be beneficial for long-term soil health and soil fertility.

Soil microbial activity can be influenced by direct resource provision or by changes in the soil abiotic environment (Cookson et al., 1998; Kaneko et al., 1998). Soil biota such as earthworms may also have top-down control over soil microorganisms through direct feeding, disruption of fungal hyphae, resource competition, or mechanical disturbance; whereas soil mites may positively or negatively influence soil microbial activity by altering the microbial population and turnover (Kaneko et al., 1998). We showed that EFB application enhanced soil microbial activity through direct resource provision, as well as through increasing soil fauna feeding activity. This finding suggested that the enhanced soil fauna feeding activities under EFB application may have produced readily-available nutrients for microbial utilization.

4.3 Effects of EFB application rate

The response of most soil abiotic properties, soil biota and ecosystem function indicators were independent of the application rate of EFB treatments. Specifically, soil pH, soil aggregate stability, earthworm biomass, total soil mite density, and microbial activity were not statistically different between the Low-EFB, Medium-EFB, and High-EFB treatments. Few soil ecosystem indicators showed higher enhancement under the Low-EFB treatment than the Medium-EFB treatment or the High-EFB treatment. For example, the soil mite Suborder of Oribatida under the Low-EFB treatment was higher than the Medium-EFB treatment. Similarly, the soil fauna feeding activity under the Low-EFB treatment was higher than the High-EFB treatment. The application rate of the Low-EFB treatment was similar to standard operations in the oil palm industry, whereas Medium-EFB and High-EFB treatments represented alternative application rates of EFB. These results suggest that better soil abiotic properties, soil biota and ecosystem functions may be enhanced by the low, industry standard EFB treatment without the need to increase the application rate.

5. CONCLUSIONS

Applying a major oil palm industrial residue empty fruit bunch (EFB) for 15 years in Indonesia improved soil abiotic properties and increased soil detritivore mites abundance, soil fauna feeding activity, and soil microbial activity. EFB affected soil biota and function indicators by providing resources, rather than through altering soil abiotic properties. The effects of EFB application on most of measured soil parameters were independent of the application rate. These results suggest that the current practice of EFB application in some oil palm plantations has a high potential to enhance soil abiotic properties, soil biota and functions. We highlight this practice as a promising approach to enhance sustainable development of oil palm.

6. AUTHORS' CONTRIBUTIONS

All authors conceived the ideas and designed methodology; HHT collected the data and wrote the manuscript; HHT and LH analysed the data. All authors contributed critically to the drafts and gave final approval for publication.

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9. FIGURE CAPTION

Figure 1. Soil pH (a), soil moisture (b), aggregate stability (c), bulk density (d), soil organic carbon (e), and soil total nitrogen (f) under Chemical fertilizer treatment (Chemical fertilizer), Low-EFB treatment (Low-EFB), Medium-EFB treatment (Medium-EFB), and High-EFB treatment (High-EFB). The filled circles indicate treatment means and bars indicate standard errors. Different letters indicate significant Tukey's HSD differences between treatments. EFB: empty fruit bunch. ns: non-significant difference.

Figure 2. Earthworm biomass (a), soil mite density (b), soil fauna feeding activity (c), and soil microbial activity (d) under Chemical fertilizer treatment (Chemical fertilizer), Low-EFB treatment (Low-EFB), Medium-EFB treatment (Medium-EFB), and High-EFB treatment (High-EFB). The filled circles indicate treatment means and bars indicate standard errors. Different letters indicate significant Tukey's HSD differences between treatments. In (b), the lower case letters indicate statistical differences in the total density of soil mites, while the upper case letters indicate the differences in the density of Oribatida. EFB: empty fruit bunch.

Figure 3. Structural equation modelling for the effects of EFB application on soil abiotic properties, soil biota, and functions. The models provided the best fit to the data and were well supported (Fisher's C=35.02, df=38, *P*-value=0.61). The number besides each arrow indicates path coefficient (standardized partial regression coefficient; +: positive effect, –: negative effect). The significance of the effects were determined by the *P*-value (*, ** and *** indicate that F-value differs significantly from 0 at P = 0.05, 0.01, and 0.001). The arrows indicate unidirectional relationships between the variables. Black arrows indicate positive effects, grey arrows indicate negative effects, and dashed arrows indicate

non-significant relationships. Arrow widths are proportional to the path coefficients. The conditional coefficients of determination (r^2) for each endogenous variable is reported.

Figure 4. Log-transformed soil microbial activity as CO_2 concentration (mg C-CO_2 kg^{-1} soil day^{-1}), as a function of square-root transformed soil fauna feeding activity (%).Points are measured values from Chemical fertilizer treatment, Low-EFB, Medium-EFB, and High-EFB treatments. The line indicates a fitted linear regression model (y=2.99+1.31x, R^2=0.13, *P*-value<0.001). The grey band indicates 95% of the confidence interval. Linear mixed effects model including random effects of treatment plot nested within replicate block showed significant effects of soil fauna feeding activity on soil microbial respiration ($F_{1,70}$=8.75, *P*-value=0.0042).