Impact of soil collar insertion depth on microbial respiration measurements from tropical peat under an oil palm plantation

S.F. Batubara¹, F. Agus², A. Rauf¹ and D. Elfiati³

¹ Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia
 ² Indonesian Soil Research Institute, Bogor, Indonesia
 ³ Faculty of Forestry, Universitas Sumatera Utara, Medan, Indonesia

SUMMARY

Carbon dioxide (CO₂) emissions from peat are commonly measured using closed chambers, using a collar insertion depth of 5–20 cm. However, measured emission values at these depths are likely to be a mixture of root (autotrophic) and microbial (heterotrophic) respiration. Using a deeper collar insertion depth of 60 cm may minimise the influence of root respiration. We compared CO₂ fluxes measured from six shallow (20 cm insertion depth) and six deep (60 cm insertion depth) collars. The collars were installed permanently in a mature (25-year old) oil palm plantation; 450 cm from the base of individual palm trees. Carbon dioxide fluxes were measured in the field bi-weekly from May to October 2017 using an Infrared CO₂ Gas Analyser (IRGA). For each measurement date, CO₂ fluxes from the shallow collars were consistently higher than from the deep collars. On a daily basis, the mean CO₂ emission rate from the deep collars was 448 ± 25 mg m⁻², 29 % lower than mean emissions recorded from the shallow collars (634 ± 30 mg m⁻²). The significant difference (p = <0.001) in emissions between the different insertion depths implies that even at a 450 cm distance from the tree (approximately mid-way between fully mature palms) root respiration influences CO₂ fluxes. This suggests that in order to minimise overestimation of soil CO₂ produced via microbial respiration, root trenching or the use of deep insertion collars is important. Emissions from the deep collars may better represent soil microbial respiration than shallow collars.

KEY WORDS: closed chamber, CO2 emissions, IRGA, microbial respiration, root respiration

INTRODUCTION

Peat forest conversion to cultivated land in the tropics, which is usually associated with drainage, has resulted in increased carbon dioxide (CO₂) emissions (Jaenicke *et al.* 2010). The soil respiration process, which is a combination of microbial (heterotrophic) and root (autotrophic) respiration, plays an important role in the biosphere carbon (C) cycle (Ojanen *et al.* 2012).

Hooijer *et al.* (2010) have estimated that global CO_2 emissions from drained tropical peatlands are 355–855 million Mg yr⁻¹. Furthermore, Joosten (2007) has argued that CO_2 emissions from peatlands in Indonesia are the highest in the world, at approximately 500 million Mg yr⁻¹. Drained boreal peatlands are also an important source of CO_2 emissions; for example, emissions from Russian peatlands are the second largest globally, at 139 million Mg yr⁻¹.

CO₂ is produced during both plant (autotrophic) and microbial (heterotrophic) respiration, which occurs during the decomposition of litter and soil organic matter (SOM). Plant respiration can be separated into above- and below-ground components, with below-ground plant respiration generally equivalent to root respiration (Luo & Zhou 2006). The IPCC (2014) illustrates C flow paths from drained peat as consisting of CO₂ from heterotrophic soil respiration (but not from autotrophic respiration); methane (CH₄) from anaerobic decomposition; CO₂ and CH₄ from peat fires; CO₂ and CH₄ from soluble organic and inorganic C, and particulate organic C. Methane emissions are negligible under drained peat, except from drainage canals. Meanwhile, C sequestration occurs into plant biomass through photosynthesis. Part of the plant biomass forms litterfall and dead roots and the changes in C stock in the biomass and plant litter are normally measured separately in greenhouse gas inventories (IPCC 2006).

Net emissions of greenhouse gases from an ecosystem are the result of emissions from the soil surface, and uptake through photosynthesis (Schrier-Uijl *et al.* 2010). In the eddy covariance technique, the measured CO_2 flux represents net ecosystem emissions by taking into account the amount of CO_2 removed by photosynthesis, because the gas concentration is normally measured above the plant canopy (Schrier-Uijl *et al.* 2010). However, when closed chambers are used for measuring CO_2 fluxes, the measured soil CO_2 is a mixture of heterotrophic and root respiration (Jauhiainen *et al.* 2012).

Data on C emission rates from peatlands vary greatly due to the high variation in the physical, chemical and biological properties of the peat soil, as well as the variety of research methods employed (Agus & Sarwani 2012). CO_2 emissions from root respiration can confound estimates of emissions from microbial decomposition. Handayani (2009) showed that about 30 % of emissions from oil palm plantations are from root respiration. Similarly, Dariah *et al.* (2014) showed that in Jambi Province,

Indonesia, CO₂ emissions from peatlands under 5and 15-year-old oil palm, were 38.2 ± 9.5 and $34.1 \pm$ 15.9 Mg ha⁻¹ year⁻¹, respectively, at points relatively unaffected by root respiration (> 3 m from the base of the palm tree). However, at a distance of < 2.5 m from the palm tree base, total soil respiration increased due to the increase of root related respiration. This study estimated that heterotrophic respiration (expressed as CO₂) contributed 86 % of the 44.7 ± 11.2 Mg ha⁻¹ yr⁻¹ weighted surface flux in the 5-year old plantation, and 71 % of the 47.8 ± 21.3 Mg ha⁻¹ yr⁻¹ in the 15-year old plantations.

Understanding the spatial distribution of oil palm roots in a plantation, and positioning soil flux collars in areas with minimal root growth, may help to minimise the contribution of root respiration to overall CO₂ fluxes. Sinuraya (2010) found that in tropical peat soils with a drainage depth of 60-75 cm, oil palm roots were most abundant 30-45 cm below the soil surface. Moreover, Marwanto et al. (2013) demonstrated that the density of palm roots 0-30 cm below the soil surface of a 15-year old oil palm plantation was negligible (<0.2 g dm⁻³) \geq 4 m from the centre of palm tree base. Marwanto & Agus (2014) found that the density of roots of < 5 mmdiameter decreased with increasing distance from the palm tree, and that the density of fine roots (< 2.5 mmin diameter) was correlated with soil respiration.

Microbial activity may also be influenced by root density. Several reviews state that soil influenced by a high root turnover is preferred by many microbes, relative to bulk soil (e.g. Kuzyakov 2000, Paterson 2003). Subke *et al.* (2004) and Hamer & Marschner (2005) have also demonstrated that microbial activity increases in response to high concentrations of nutrients and labile C, and is influenced by root exudates. However, the mechanism of CO_2 emissions from the soil to the atmosphere is complex and influenced by interacting environmental factors (Handayani 2009).

There is a need to distinguish between soil and root respiration, and to understand how to minimise the influence of root respiration on CO_2 flux

measurements of tropical peat soils. The objective of this study is, therefore, to evaluate the effect of soil flux collar insertion depth on measurements of microbial respiration from tropical peat under an oil palm plantation. We aimed to minimise the contribution of root respiration in measurements of CO_2 flux by preventing roots from entering the collars with deep (60 cm) insertion, and compared the results with measurements using shallow (20 cm) insertions. The ability to exclude most root respiration from CO_2 flux measurements using deep collars may increase the accuracy of CO_2 peat emission estimates and could potentially contribute to improved emission factors for this ecosystem.

METHODS

Site description

This study was conducted in Labuhan Batu Selatan District (2° 0' 48" N, 100° 16' 7" E), North Sumatra, Indonesia. In this study site, the peatland forest was cleared in 1991 for transmigrants from Java island who began planting oil palm in 1992. Currently, the oil palm is about 25 years old and is close to the age of replanting. Thus, conditions likely represent the maximum size that oil palm root systems will reach.

Peat characteristics

Soil samples for peat property analyses were taken from the traffic (TI) and palm frond pile (FI) interrows (between rows of oil palms) from the surface (0-20 cm), and subsurface (20-40 cm and 40-60 cm)layers, using minipits (Figure 1) that were 50 cm long \times 50 cm wide \times 60 cm depth. Each sample was thoroughly mixed and cleaned from root debris, oven dried at 50 °C for 24 hours and analysed for chemical characteristics. For soil bulk density analysis, the known volume of samples from the Ejkelkamp peat auger tube (200 ml for each 20 cm soil depth increment) was transferred into plastic bags for transportation from the field to the laboratory. The soil analyses were conducted at the Soil Laboratory of Indonesian Soil Research Institute (ISRI) in Bogor, Indonesia.

Soil chemical properties were analysed, including: soil pH_{H2O_2:5} using a pH glass electrode, soil C content using the Walkley and Black method, total nitrogen (N) was determined using the Kjeldahl distillation method, and plant available phosphorus (P) was determined with the Bray and Kurt extraction technique (IAARD 2012). Analyses of soil physical properties included: peat maturity, bulk density and ash content. Peat maturity was determined qualitatively by visual interpretation: peat samples were squeezed by hand; maturity was evaluated based on the remaining fibrous peat in hand: > 75 %for fibric (immature), between 25 and 75 % for hemic (moderately mature), and < 25 % for sapric (mature) peat (Agus et al. 2011). Bulk density was determined in the laboratory using the gravimetric method. Dry weight of the samples was determined after oven drying until the samples reached a constant weight (105 °C for 5 to 7 days, depending on the moisture content and the number and amount of the samples; Agus et al. 2011).



Figure 1. Schematic representation of closed chamber (collar) and minipit positions along the frond pile inter-rows and traffic inter-rows under mature oil palm trees. Schematic is not to scale to improve clarity.

CO₂ flux measurements

We installed opaque collars (21.7 cm diameter PVC pipes) permanently in the ground during the course of the six month flux measurement period. The "deep collars" (80 cm in length) were driven vertically into the ground to a depth of 60 cm, which left the top 20 cm above ground as headspace for capturing the emitted CO₂. The "shallow collars" (40 cm long) were driven 20 cm into the ground to leave 20 cm of headspace. Previous studies have demonstrated that oil palm roots in peatland are most abundant at a depth of < 45 cm (Sinuraya 2010; Marwanto et al. 2013); thus, the use of the 60 cm long collar is expected to cut most roots and minimise the influence of root respiration. However, the cut roots remained inside the collars and likely became a substrate for microbes, who released CO₂ as heterotrophic respiration.

The lower edges of the 0.4 cm thick PVC collars were sharpened to minimise peat soil compaction during collar installation and to assist cutting of fine roots. A small shovel was used if installation of the collars were obstructed by roots. The collars were closed during measurements with a PVC pipe stopper "hood", equipped with tubing for circulating gas from the headspace to the Infrared CO₂ Gas Analyser (IRGA), and vice versa, using a pressure pump, and an air vent to release gas from the headspace due to increased pressure (Madsen et al. 2009). The hood (4 cm long) was portable and was placed tightly on top of the collar during each measurement. A mercury water thermometer was installed at the top of the hood with an airtight rubber fitting to measure air temperature in the headspace in each collar during measurements.

Two collars, one of each length (deep/shallow), were installed adjacent to each other in six locations in the field site, allowing pairwise comparisons between results from the different depths. The collars were placed 450 cm from the centre of the base of a palm tree (about mid-point between the palms) and the distance between the collars was about 75 cm (Figure 1). Three pairs of collars were placed in the palm frond pile inter-row (FI; where palm fronds were stacked after removal from the trees) and three pairs in the traffic inter-row (TI; kept clear to allow movement around the plantation). The collar positions were not directly fertilised, as fertilisation for oil palm is restricted to a 2 m radius around the palm trees.

The collars were installed in early May 2017 and left in the ground for the duration of the research. Biweekly field CO₂ flux monitoring commenced two weeks after collar installation. Twelve measurements per collar were completed during the six-month monitoring period (May to October 2017), conducted between 07:00 am and 10:00 am. Measurements of CO₂ concentration in the headspace of each collar were taken using an IRGA (LiCor 820 Model); CO₂ concentration was recorded every second for 150 seconds. Readings from the first 10–20 seconds were usually unstable and hence were excluded from analysis. The linear relationship between CO₂ concentration and time were accepted when the R² was \geq 0.9.

Soil CO₂ flux was calculated using the following equation (Madsen *et al.* 2009):

$$f_c = \frac{Ph}{RT} \frac{dC}{dt}$$
[1]

where:

 $f_c = CO_2 \text{ flux (}\mu\text{mol }\text{m}^{-2} \text{ s}^{-1}\text{)}$

- P = Atmospheric pressure (kPa) in the headspace, based on average reading from the IRGA
- h = Chamber headspace height (cm)
- $R = \text{Gas constant} (8.314 \text{ Pa m}^3/\text{K/mole})$
- T =Air temperature (°K) in the headspace
- dC/dt = The slope (change in CO₂ concentration over time).

Soil environment

The water table was monitored twice every month, starting in December 2016, and at the same time as CO_2 flux measurements from May to October 2017, using piezometers (5 cm diameter, 2 m long perforated PVC tubes driven vertically into the

ground within 75 cm of the collars). Soil temperature was monitored 10 cm away from each collar at a depth of 5 cm, and air temperature was monitored 90 cm above the soil surface. All temperature measurements were made using mercury water thermometers at the same time as the gas flux measurements. Monthly rainfall was recorded in the 1st Division (Block C.6) Teluk Panji Plantation, approximately 2 km from the study site (November 2016 to October 2017).

Statistical analyses

A paired sample t-test was used to test for differences in CO₂ fluxes between the deep and shallow collars, involving 72 pairs (six locations \times 12 measurements). The relationship between CO₂ flux and each environmental variable was analysed using simple linear regression models and Pearson correlation. SPSS Version 20 (IBM Corporation 2011) was used for the statistical analyses.

RESULTS

Peat characteristics

Data of peat physical and chemical properties at the surface (0-20 cm) and subsurface (20-40 and 40-60 cm) layers are presented in Table 1. Mean

moisture content (calculated across all soil depths) was 77 \pm 8 %; moisture content generally increased with depth (Table 1). Peat thickness was 100–200 cm and was sapric (mature) at all depths. Mean bulk density was 0.15 g cm⁻³, and C content by weight was 371 g kg⁻¹, which is typical for mature peat (Agus *et al.* 2011). Total N content was high (12 \pm 2.4 g kg⁻¹), and available P was low (9.5 \pm 2.7 mg kg⁻¹). The soil was acidic (pH 3.9 \pm 0.04), and contained a high ash content in the upper layer, and moderate to low ash content in the lower layer (using the criteria of IAARD 2012).

Variations in rainfall, water table, and soil temperature

The rainfall pattern near the research area from November 2016 to October 2017 is presented in Figure 2. The highest monthly rainfall occurred in December 2016 (425 mm), while lowest rainfall occurred in June 2017 (56 mm). The months June, July and February 2017 had monthly rainfall of <100 mm, and were, therefore, considered as dry months. Average monthly rainfall between November 2016 and May 2017 was 182 mm, while average monthly rainfall during the CO₂ flux measurement period between May and October 2017 was somewhat lower (154 mm). Mean water table was -42 cm during the CO₂ flux measurement period

Table 1. Peat properties at different depths, from samples in the traffic interrow (TI) and the palm frond interrow (FI). SD = Standard deviation; BD = Bulk density.

Soil sample	Moisture content (% by weight)	Peat maturity	BD (g cm ⁻³)	Carbon content (g kg ⁻¹)	Nitrogen content (g kg ⁻¹)	Available P (mg kg ⁻¹)	рН	Ash Content (g kg ⁻¹)
TI 0–20 cm	70	saprist	0.14	362	11	10.9	3.9	309
TI 20–40 cm	71	saprist	0.13	385	16	12.7	3.9	173
TI 40–60 cm	85	saprist	0.14	383	12	7.8	3.9	104
FI 0–20 cm	67	saprist	0.23	356	13	6.2	4.0	213
FI 20–40 cm	81	saprist	0.17	360	10	12.0	3.9	61
FI 40–60 cm	86	saprist	0.11	382	0.9	7.3	3.9	89
$Mean \pm SD$	77±8	-	0.15 ± 0.04	371±13	12±2.4	9.5±2.7	3.9±0.0	158±93



Figure 2. Rainfall distribution (mm) from November 2016 to October 2017, recorded at the 1st Division (Block C.6) Teluk Panji Plantation, approximately 2 km from the study site.

(May to October 2017), which was somewhat deeper than during December 2016 to October 2017 (-35 cm). Water table varied between -5 and -63 cm during the year; water tables were relatively deep in June, and from August to October, and shallower in December 2016 to January 2017 (Figure 3). Mean daily soil temperatures ranged from 23.8 to 28.2 °C during the measurement period.

CO₂ fluxes

CO₂ fluxes in the shallow (T0) and deep (T1) collars showed large variation during the measurement period (Figure 3). The highest recorded daily CO₂ flux in the shallow collars was 807 mg m⁻² day⁻¹ (24 July 2017) and the lowest was 417 mg m⁻² day⁻¹ (6 August 2017). The highest CO₂ flux recorded in a deep collar was 631 mg m⁻² day⁻¹ (on 24 June 2017) and the lowest was 269 mg m⁻² day⁻¹ (6 August 2017, lower than the shallow collar; Figure 3). Mean CO₂ flux in the shallow collars was $634 \pm 30 \text{ mg m}^{-2} \text{ day}^{-1}$, and 448 ± 25 mg m⁻² day⁻¹ in the deep collars (Table 2). Mean flux values for the six month period (May to October 2017) in the shallow collars was significantly greater than that of the deep collars (p value = <0.001, Table 2). On average, emissions measured using the deep collars were 29 % lower than when measured using the shallow collars.

There was no correlation between CO₂ flux and water table (r = 0.0168; r = -0.2027; Figure 4), and no correlation between CO₂ flux and soil temperature (r = 0.0599; r = 0.1452; Figure 5) for the shallow and the deep collars, respectively.



Figure 3. Water table (WT) fluctuation from December 2016 to October 2017, and carbon dioxide (CO₂) fluxes from May to October 2017 for the shallow (T0) and the deep (T1) collars.

Collar insertion	Mean daily (mg m ⁻² day ⁻¹) ± SE	$\begin{array}{l} \text{Mean annual} \\ (\text{Mg ha}^{-1} \text{ yr}^{-1}) \\ \pm \text{SE} \end{array}$	N	Correlation	t	P-value
Shallow (20 cm)	634 ± 30	55.5±2.6	72	0.83	11.1	< 0.001
Deep (60 cm)	448 ± 25	39.3±2.2	72			

Table 2. Paired sample t test of carbon dioxide (CO₂) emissions (Mg ha⁻¹ yr⁻¹) from the shallow and the deep collars. SE = standard error.



Figure 4. Relationship between water table and carbon dioxide (CO_2) flux for the shallow (T0) and deep (T1) collars (r = Pearson correlation).

DISCUSSION

Effects of seasonal variation on CO₂ fluxes

We conducted the measurement of CO₂ fluxes from May to October 2017, which represents wet months (May and August), moist months (September and October), and dry months (June and July) (Figure 2). Total monthly average rainfall during the 6 month period of this research (154 mm) was slightly lower than the annual monthly average (182 mm). This implies that the flux values reported from this study are likely to be typical for an average year. The highest CO₂ flux occurred in the dry months on 24 June and 24 July (Figure 3) during which time the water table was not particularly deep (Figure 2). Previous studies of tropical and subtropical wetlands have shown a strong seasonal (wet and dry) influence on soil CO_2 fluxes, but in this study, we showed that CO₂ fluxes were not apparently influenced by water



Figure 5. Relationship between soil temperature and carbon dioxide (CO₂) for the shallow (T0) and deep (T1) collars (r = Pearson correlation).

table depth. Jauhiainen et al. (2005, 2016) and Schedlbauer *et al.* (2010) reported that CO_2 fluxes were greater during the dry season and lower during the wet season. Seasonal variation in precipitation influences the hydrological status of tropical peatlands and is the primary factor that influences trends in CO₂ fluxes (Hashimoto et al. 2007) by affecting water table depth and soil moisture status (Jaenicke et al. 2010). During the dry season, the water table usually falls well below the peat surface (to -120 cm) and this generally increases CO₂ emissions (Jauhiainen et al. 2005, 2016). However, in the current study, water tables did not drop below -50 cm from the soil surface, except on the 11th of June when it reached -63 cm, due to the shallow drainage system in this plantation. The reduced degree of drainage and absolute water table depth relative to previous studies may explain the difference in findings, and the lack of relationship between CO_2 flux and water tables in this study (Figure 4). The small dataset in the study may have also contributed to the lack of observed relationship. Several other studies have reported similar results (e.g. Nieveen *et al.* 2005, Kechavarzi *et al.* 2007, Berglund *et al.* 2010) where no correlation was found between water table and CO_2 flux.

Laiho (2006) has emphasised the complexity of the dynamics of water table, temperature, and precipitation that leads to uncertain relationships between, for example, water table and CO₂ flux, which may exist if the experiment had been conducted controlled under environmental conditions. The relationship between CO₂ fluxes and temperature also showed no correlation (Figure 5), whether for shallow or deep collars. This is to be expected, as in tropical and subtropical forest ecosystems, the influence of temperature on root growth and soil microbial activities is low (Zheng et al. 2009).

Effects of root respiration on CO₂ fluxes

The limitations of this study are that CO_2 flux measurement was conducted only every two weeks for a six month period. However, there may be some merit of presenting the results in term of mean annual emissions for direct comparison with the results of other studies.

Mean annual CO₂ emissions from the deep collars (calculated across all measurements for the entire year) of 39.3 ± 2.2 Mg ha⁻¹ yr⁻¹ were significantly lower than that of the shallow collars (Table 2). We attribute this difference to the additional CO₂ emitted from root respiration that was measured in the shallow collars. Within and below the shallow collars there may have been a substantial density of roots whose respired CO₂ moved upward and entered the collars (Jauhiainen *et al.* 2012, Dariah *et al.* 2014). In contrast, under the deep 60 cm long collars, root density is expected to be minimal as demonstrated by Sinuraya (2010) and Marwanto *et al.* (2013), although there was a potential for more CO₂ emissions caused by microbial decomposition of the cut roots held within the deep collars than within the shallow ones. In addition, with water tables mostly above -50 cm during the course of the study period (Figure 3), root growth and respiration are likely to be restricted to the top layers of the soil above the water table (Boggie 1972).

Previous studies have reported heterotrophic respiration values of 38.2 ± 9.5 and 34.1 ± 15.9 Mg ha⁻¹ yr⁻¹ under 5- and 15-year old oil palm plantations, respectively (Dariah *et al.* 2014), and 46 ± 30 Mg ha⁻¹ year⁻¹ from a15-year old plantation (Marwanto & Agus 2014). Our findings are within the range of these studies, but are much lower than findings by Jauhiainen et al. (2012) of 94 Mg ha⁻¹ yr⁻¹ under an Acacia plantation with a mean water table depth of -80 cm. As Acacia is a N-fixing tree, soil N is therefore likely to be higher under Acacia, which could stimulate a higher rate of microbial decomposition (Brockwell et al. 2005). Furthermore, our findings are lower than that reported by Husnain et al. (2014); 56 to 67 Mg ha⁻¹ yr⁻¹, whose research was on fibric peat (rather than sapric peat) and under relatively open canopies of rubber, oil palm, degraded forest, and shrub, all affected by the same drainage canal and all less than 2 km distance from each other. They found no significant differences in CO₂ emissions under different land covers.

The contribution of autotrophic respiration to total CO₂ fluxes seems to be dependent on root distribution (determined by the type and age of plants, the insertion depth of collars, and measurement position), and the environmental conditions that govern plant physiology. Handayani (2009) conducted a field experiment (positioning oil palm roots within a closed chamber) in Aceh Province, Indonesia, to show that approximately 30 % of total soil CO₂ emissions came from root respiration. Other studies report root respiration contributions of 13-40 % (Schindlbacher et al. 2010), 21 % (Jauhiainen et al. 2012), and 46 % (Melling et al. 2007). Interestingly, Jauhiainen et al. (2012) found that root respiration contributed to 80 % of CO₂ flux in Acacia plantations, when measured

0.5 m from the trees, but was negligible at points 1.3 m away from the trees. Dariah *et al.* (2014) also reported higher autotrophic respiration at points closer to the palm trees, but negligible root respiration > 3m away from the trees.

In our study, the 25-year old oil palm has reached its maximum size, and it was clear that CO₂ flux measurements using deep collars produced results 29 % lower than those using shallow collars, due to the contribution of root respiration in the latter. With the deep 60 cm collar, it is very likely that most root respiration is excluded, due to root cutting when the collars were inserted into the ground and that practically no root activity below 60 cm from the surface occurred due to the relatively shallow water table (above -50 cm on most measurement days). The significant difference in CO₂ emissions between the different collar depths implied that, for this 25-year old plantation, the influence of root respiration extended to the mid-point between the oil palm trees, suggesting the necessity for root trenching or the use of deep collars to minimise overestimation of soil CO₂ emissions due to root respiration.

ACKNOWLEDGEMENTS

We appreciate the anonymous reviewers for their constructive suggestions on this manuscript. We would like to thank the Abdi Budi Mulia Plantation company and the people of Desa Teluk Panji III and Teluk Panji II, Labuhan Batu Selatan District, North Sumatra, Indonesia for their support during the field work. We thank the Assessment Institute of Agricultural Technology, and North Sumatra University for laboratory facilities. Grateful thanks are also addressed to other parties who cannot be mentioned individually for giving us their hands to accomplish this work. Special thanks to Dr Eleanor Warren-Thomas, University of York for language editing and useful inputs to the manuscript. This research was funded by the Indonesian Agency for Agricultural Research Development (IAARD). All

four authors were directly involved in the research design and discussion of the results. The first and the second authors were responsible for data analysis, manuscript writing and addressing reviewers' comments, and revising the manuscript. The third and the fourth authors provided inputs for revisions.

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Submitted 27 Aug 2018, final revision 08 Mar 2019 Editor: David Wilson

Author for correspondence:

Ms Siti Fatimah Batubara, Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia. Permanent address: Assessment Institute of Agricultural Technology, Medan, Indonesia. Email: sifa.cha@gmail.com