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Research Article

The contribution of organic acid on heterotrophic CO_2 flux from tropical peat: a trenching study

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Abstract

Quantification of CO_2 flux from peat has been studied with various methods of measurement and data analysis. Several studies have applied regression analysis to assess carbon flux from tropical peatland as a function of groundwater level. Such an analysis simplified the complex nature of peat decomposition, which involved microbial activities. The study was conducted at Buatan Village, Siak Indrapura Regency, Riau Province, Indonesia. Soil sampling was done every month for a year observation, from July 2018 to June 2019. This study aimed to comprehend CO_2 production from the respiration of heterotrophic components (Rh-CO_2) as a function of soil properties determined by soil pH, N-NH_4, N-NO_3, available P, exchangeable-K, C-organic acids, and environmental factors that are determined by soil water content, and groundwater level. The study applied trenching experimentation to quantify Rh-CO_2 flux by first removing plant roots from the trenching plot. The CO_2 flux and groundwater level were measured for five consecutive days each month for a one-year period. Multiple regression analysis was performed to determine the main determinant for the Rh-CO_2 flux. The results showed that seasonal fluctuation of Rh-CO_2 flux, negatively correlated with available P (p = 0.037), and positively (p = 0.018) with C-substrate as C-organic acids but not with either of the speciated ones as acetic, lactic, citric, malic, nor oxalic acids. More specifically, the C-organic acids were found as the main determinant factor (p = 0.039) affecting the Rh-CO_2 flux.

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Introduction

Peatland ecosystem is formed and developed from the deposition of organic matter, which is influenced most by the dynamic hydrological conditions that form aerobic and anaerobic zones. Hence, the depth of each zone is determined by groundwater level. With increasing depth of the aerobic zone, when it is drained, it is allowed to the faster decomposition and conversion of organic matter into CO_2 due to oxygen intake into the peat layer. Several researchers interpreted CO_2 loss from a peatland as a function of groundwater level, and most of them reported positive correlations (Carlson et al., 2015; Wakhid et al., 2017). Nevertheless, the model function is not always the case as those reported by Melling et al. (2013a) and Sumawinata et al. (2014), in which decreasing groundwater level is reported not correlated with CO_2 loss. Peat oxidation is an enzymatic process that is involving and catalyzed by microbial activities. Groundwater level fluctuation affected oxygen availability, but the level of groundwater cannot be used as an indicator of soil moisture that influences pores continuity in peatland. Therefore, soil moisture of the upper peat layers may be a better determinant factor for CO_2 production than groundwater level (Melling et al., 2013a; Moyano et al., 2013).

Soil respiration is the largest contributor (60-90%) of CO_2 released to the atmosphere from terrestrial ecosystems (Longdoz et al., 2000). These contributions come from two respiration processes, i.e. autotroph component (Ra), such as roots and other autotrophic organisms, and heterotroph component (Rh), decomposition of organic matter by microbes that control nutrients storage and dynamics. Quantification of Rh-CO_2 can be done by various methods, such as determination of CO_2 flux difference based on spatial variation of soil respiration by considering the root distribution as a function of distance from the tree (Dariah et al., 2014; Matysek et al., 2018) and trenching (Comstedt et al., 2011: Savage et al., 2013; Wakhid et al., 2017). Trenching method is widely used to estimate the Rh-CO_2. However, most of the trenching is made without removing plant roots in the trenching plot and ignoring the maximum height of the ground water level during the dry season, especially in tropical peat where the groundwater level is very fluctuated and can affect groundwater movement. In this regard, water is essential for the entire intra- and extracellular reactions that supporting microbial life and solutes movement.

Soil microbes, as a determinant of plant diversity and productivity, affect the quality and quantity of C inputs to the soil (De Deyn et al., 2008). The availability of C as a substrate for microbial activity (Kuzyakov and Gavrichkova, 2010; Blessing et al., 2016) and supporting environmental factors, such as temperature and moisture (Gomez-Casanovas et al., 2012; Savage et al., 2013), accelerate the rate of heterotrophic microbial activity, hence C is released into the atmosphere as CO_2.

Several studies have determined that soil temperature and groundwater level are the main determinants for CO_2 flux from peatland and soil respiration was frequently predicted using a linear regression model incorporating one of them, while the availability of substrate for microbes was often neglected. In fact, soil respiration is influenced by various factors that are interconnected with each other. Understanding the Rh-CO_2 flux changes in an ecosystem is crucial to determine its contribution to soil respiration. We conducted multiple regression analysis by including several soil properties and environmental factors as independent variables to figure out which one would be the main determinant of Rh-CO_2 flux from tropical Indonesian oil palm peatland based on the results of one-year trenching experiment as the objectives of the study.

Materials and Methods

Study site

The study was conducted at an oil palm cultivation area located at Buatan Village, Siak Indrapura District, Riau Province, Sumatra, Indonesia (00°42'12" E, 101°44'07" S), which are dominated by peat soils with varying peat thickness (peat depth >200 cm). According to soil taxonomy (Soil Survey Staff, 2010), the soils are classified as Haplohemists. Average monthly rainfall in the study site is quite high and characterized by a yearly bimodal pattern as it consists of two peaks of the rainy season and dry season as well (Figure 1). Rainfall events are recorded by Automatic Weather Station (AWS-Davis Vantage Pro 2 Plus). On a one-year basis, NPK (15% N - 6% P_2O_5 - 24% K_2O) fertilization was applied in two stages, whilst for Cu-EDTA, Zn-EDTA, and borate as well as liming with $CaCO_3$ were carried out according to the recommended standards dosages that apply for oil palm cultivation in Indonesia.

Measurement of CO_2 flux and groundwater level

Soil respiration of the heterotrophic components was measured by conducting one-year trenching experimentation that was modified from Sumawinata et al. (2014). The trenching plot was constructed in May 2018, with a dimension of 400 cm length, 150 cm width, and 100 cm depth. The latter was determined based on the lowest groundwater level at the site that was -80 cm so that the depth of the trenching plot was set to 100 cm with the consideration that below this groundwater level, root growth would be hampered. After removal of the dug soil and roots, the trenching plot was enclosed with gray corrugated plastic and the soil separated from the roots debris through the sieve having 1-cm^2 were put back in. Then, three chambers and one dipwell were installed on the trenching plot (Figure 2). The chambers were made of PVC pipe with the same diameter and height of 25 cm; they were then inserted 5 cm below the peat surface. The soil and air temperature were measured with a mercury thermometer placed close to the chambers. The CO_2 flux measurements were done in the morning (at 07.00-08.30 AM, local time) and afternoon (at 01.00-02.30 PM) to represent the minimum and maximum daily respiration so that the average daily respiration of the heterotrophic components (Rh-CO_2) was obtained. Dipwell was made from PVC pipe with a diameter of 3 inches and 200 cm length equipped with a crossbar of 100 cm height for groundwater level measurement.

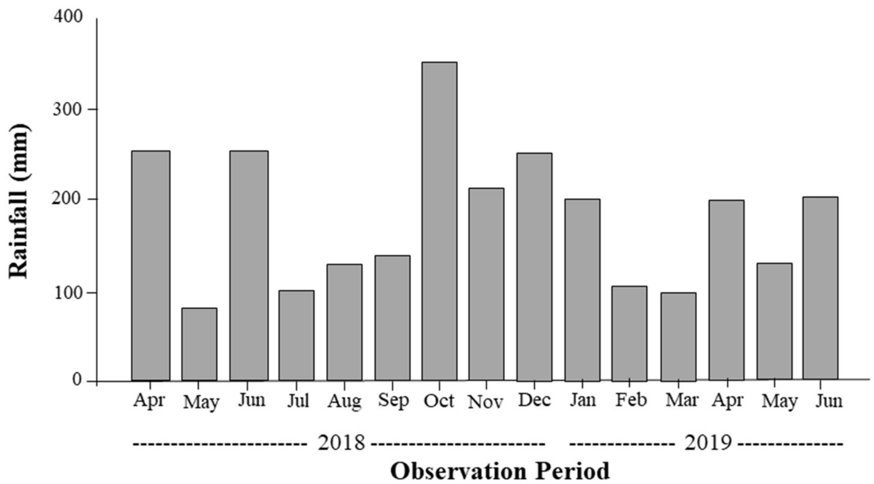

Figure 1. Monthly distribution of rainfall during the study period.

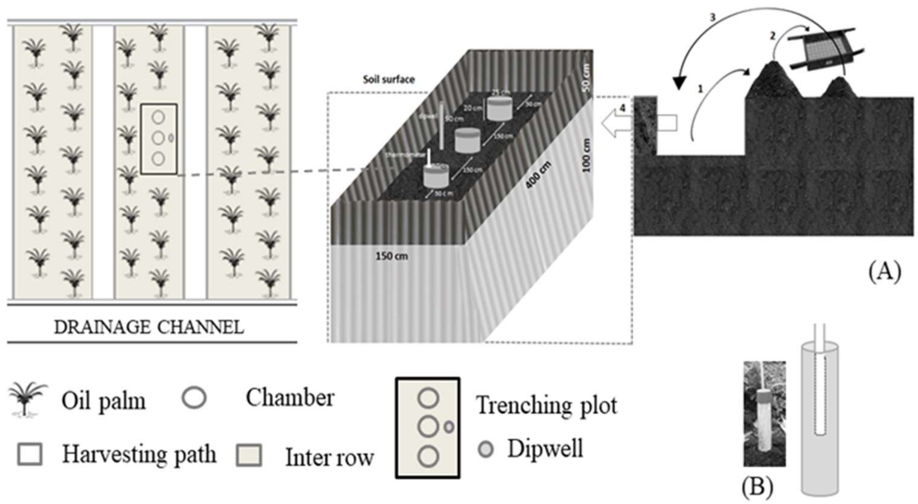

Figure 2. Layout and preparation of the trenching plot experimentation: (A)1. Removal of bulk soil from trenching plot; 2. Sieving of the dug soil; 3. Enclosement of the surfaces inside the dug plot and replacement of the sieved soil into the plot; 4. Instalment of the chambers and Dipwell; (B) Dipwell.

Measurement of CO_2 flux was conducted using Infra-Red Gas Analyzer (IRGA; LI-820, Li-Cor, Lincoln, Nebraska, USA) with a closed-flow chamber method. Soil temperature, air temperature, and groundwater level were measured simultaneously. Measurements were carried out for five consecutive days each month, from July 2018 to June 2019. The IRGA instrument was calibrated every month using soda lime as 0 ppm standard and CO_2 gas standards of 550 ppm.

The measurement of CO_2 flux began when the chamber was closed. The CO_2 from the closed chamber flowed and connected to the IRGA device. The device displays readings of the CO_2 gas concentration in every one-second interval and records the readings simultaneously during the air flow. Linear regression analysis involving time (second) as X-axis and CO_2 concentration (μmol m^{-2}) as Y-axis were performed to calculate CO_2 flux following equation (Madsen et al., 2009) as follows:

$$f_c = \frac{Ph}{RT}\frac{dC}{dt}$$

where fc is CO_2 flux (μmol m^{-2} s^{-1}), P is atmospheric pressure recorded by IRGA (kPa), h is chamber height (cm), R is gas constant (8.314 Pa m^3 ^{o}K^{-1} mol^{-1}), T is temperature (^{o}K) and dC dt^{-1} is changes in CO_2 concentration over the time or slope of the resulted linear regression line (μmol s^{-1}).

Soil sampling and laboratory analyses

Sampling of soil in the trenching plot was carried out after the completion of CO_2 flux measurements. Soil sampling was carried out at a depth of 0-20 cm using a peat auger. In the trenching plot, soil sampling for organic acids analysis was carried out at three points (according to the chamber distance) and composited; then, the total C from all organic acids of the soil sample was used as a determinant of CO_2 flux. The total C-organic acids were obtained from the percentage of C by the molecular weight of the organic acid multiplied by the concentration of the organic acid. Extraction of organic acids from soil samples was done using 0.1 N NaOH reagents (Baziramakenga et al., 1995). Organic acids speciation into malic, lactic acetic, citric, and oxalic acids were done and quantified using HPLC (Shimadzu 20A Gradient LC System with UV-VIS Detector). Soil pH was determined using SevenExcellence pH/Ion meter S500-Mettler Toledo using water as extractant, while soil nutrients were extracted using the Morgan-Wolf ($NaC_2H_3O_2.3H_2O$ + DTPA) extractant and concentration of the available N (NH_4 and NO_3) and P were determined using UV-Vis spectrophotometer (Shimadzu) and AAS (Agilent) for K. Soil water content was measured using gravimetric method.

Data analysis.

Standard error was calculated using Microsoft Excel v.10 to test data variation and illustrated using SigmaPlot. Multiple regression analysis was performed using Minitab v.17 to find out significant determinants of CO_2 flux from the relationship between CO_2 flux as a dependent variable with soil properties and environmental factors as independent variables.

Results and Discussion

The results of CO_2 flux measurements ranged from 141.7 ± 7.8 to 303.8 ± 22.7 mg C m^{-2} h^{-1} (Figure 3). The mean temperatures in the morning and afternoon were 25.40 ± 0.09 °C and 35.25 ± 0.34 °C for soil temperature, whereas for air temperature were 26.24 ± 0.20 °C and 37.74 ± 0.28 °C, respectively. The CO_2 flux during December 2018 increased and was higher than other observed periods. The high CO_2 flux in that month was not only caused by the concentration of organic acids (Figure 4) but also by rainfall (Figure 1). High rainfall can increase soil moisture. In this condition, the CO_2 production accumulated in the sub-layer of the peat that moved through the diffusion process to the peat surface, thereby increasing the measured CO_2 flux. This is in line with the results of research conducted by Clymo and Bryant (2008) and Marwanto et al. (2019). Diffusion contributed more than 99% of dissolved gas movement (Clymo and Bryant, 2008).

Heterotrophic components of soil respiration were reflected by the microbial and soil fauna activities under aerobic conditions that related to the amount of the soil carbon (Comstedt et al., 2011; Hoyos-santillan et al., 2016).

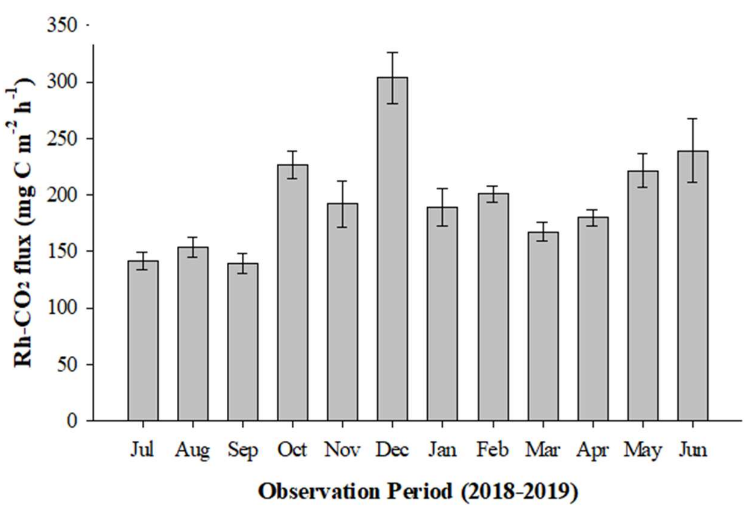

Figure 3. The dynamics of Rh-CO_2 flux

In the study site, soil organic carbon was 49.64 ± 0.49%. The availability of soil organic matter is a source of energy for soil microbes. Therefore, the turn-over of soil organic matter is determined by soil microbial biomass activity. When organic residues are added to soil, organic compounds such as organic acids will be utilized by soil microbes for activities that ultimately contribute to the carbon release, both in the rhizosphere or bulk soil. These organic compounds such as organic acids, amino acids and glucose are secreted by plant roots. The root exudates of mainly the low molecular weight acids, such as organic acids, play a key role in plant-microorganism interactions through its influence on the structure and function of soil microbial community even though the amount is very small, which is less than one percent of the total soil organic matter (Kalbitz et al., 2000). However, organic acids are the most labile and reactive fraction of the soil organic matter pool and affect many biogeochemical processes in terrestrial and aquatic environments (Kalbitz et al., 2000). In this study, C-organic acids showed positive linearity with Rh-CO_2 flux (*p* = 0.018) (Figure 4C). Root exudate, as a substrate consumed by microbes, supports microbial population in the rhizosphere and significantly contributes to the total CO_2 emission from the soil surface. Organic acids, one of the root exudate components, would be mineralized into CO_2 (about 60%) and transformed into cell biomass (about 40%) (Jones and Darrah, 1994; Jones et al., 1996).

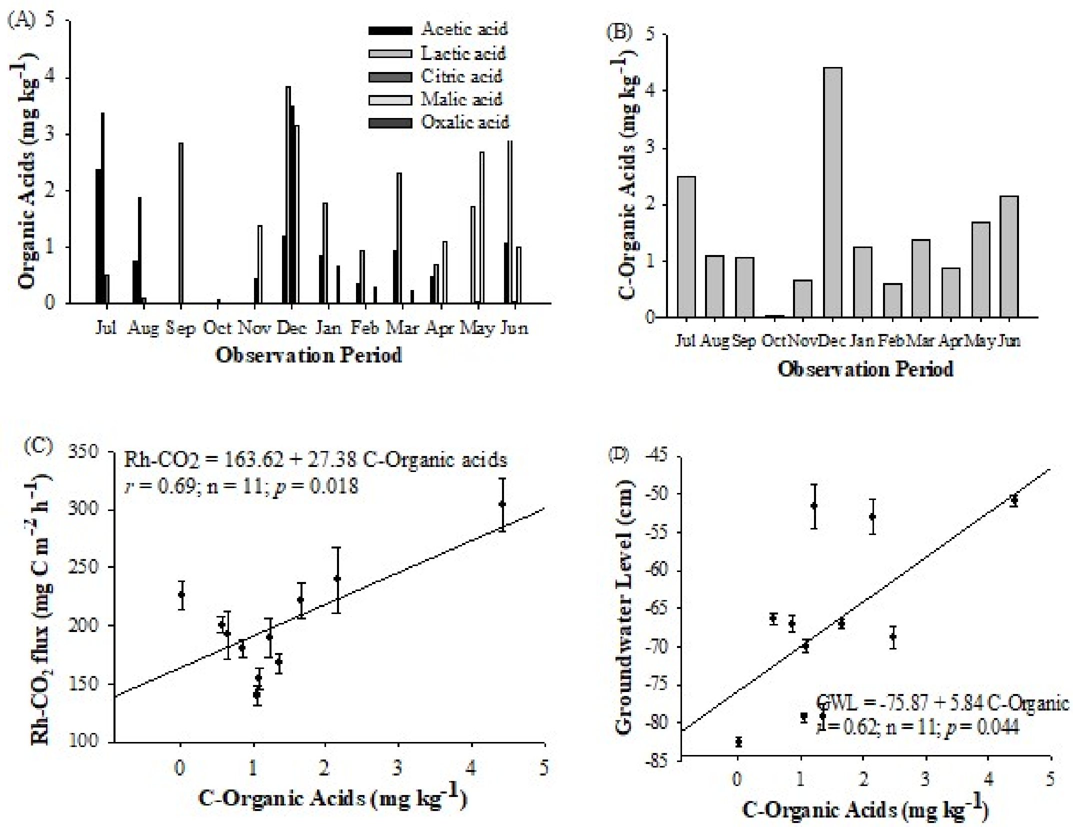

Figure 4. (A) The dynamic of organic acids; (B) The dynamic of C-organic acids; (C) Regression and correlation between Rh-CO_2 flux with C-organic acids; (D) Regression and correlation between C-organic acids with groundwater level.

Plant roots exudation and its solubility affect the concentration of organic acids. Organic acid mention was a low molecular weight compound with pKa value less than 4.0 (Manoharachary and Mukerji, 2006) so that organic acids, e.g. acetic, lactic, citric, malic and oxalic acids were freely dissolved in the environment system. Solubility of substrate occurred through diffusion, mass flow, or carried out as far as root length and are affected by intensity and volume of rainfall.

In this study, the organic acids analyzed generally derived from peat decomposition. The decomposition of the peat material produces aliphatic-carboxylic acids, benzene-carboxylic acids, and phenolic acids. The three acids have a high lignin content ranging from 57.38-73.67%, while other compounds such as protein, cellulose and hemicellulose are less than 10% (Sabiham et al., 1997; Sabiham, 2010). Organic acids are results of lignin biodegradation and sources of release C. Organic acids decompose during the observation period. Figures 4A and 4B show fluctuation of organic acids concentrations over the observation period and increased significantly in December 2018 that followed by an increase in Rh-CO_2 flux (Figure 3). In addition, a high concentration of organic acids in Juli 2018 originated from the decomposition of the remaining fine roots in the plot at the beginning of the trenching experimentation. The movement of organic acids was supported by rainfall data in the previous month (June 2018) (Figure 1). Although organic acids (acetic, lactic, and citric acids) (Figure 4A) were detected in the trenching plot, the concentration and types of organic acids had not been affected by CO_2 production yet, so CO_2 flux in July 2018 was still low. On the contrary, a high concentration of acetic, lactic, citric and malic acid was detected in December 2018 that contributed to the increase of the CO_2 flux significantly. So it can be concluded that CO_2 flux significantly correlated (p = 0.018) with the concentration of C-organic acids (Figure 4C).

The concentration of organic acids showed a positive correlation with the groundwater level (Figure 4D). The shallower the groundwater level, the higher the concentration of organic acids. Increasing the concentration of organic acids will increase microbial activity and CO_2 gas production. The degradation of carboxylates by microbes consumes H^+ and releases CO_2 and O_2 (Grams et al., 2003).

A high concentration of organic acids could acidify the soil, characterized by a decrease in soil pH. Besides soil pH, nutrient availability was substantial for microbial activity and plant respiration processes. Szczerba et al. (2009) stated that soil-N and soil-P play a significant role in the soil organic carbon mineralization rates, while soil-K contributes to the root exudation. Figure 5 shows that CO_2 flux tends to increase with the decrease of soil pH, N-mineral ($N\text{-}NH_4^+$ + $N\text{-}NO_3^-$), and exchangeable-K as well as available-P (p = 0.037), which negatively correlated with CO_2 flux. Nitrogen, phosphorus, and potassium released into the soil solution will not only be taken by microbes for the decomposition processes that increase CO_2 flux but also the probability of leaching. As a consequence of increasing N, P and K uptake, the concentration of these elements in the soil were decreasing.

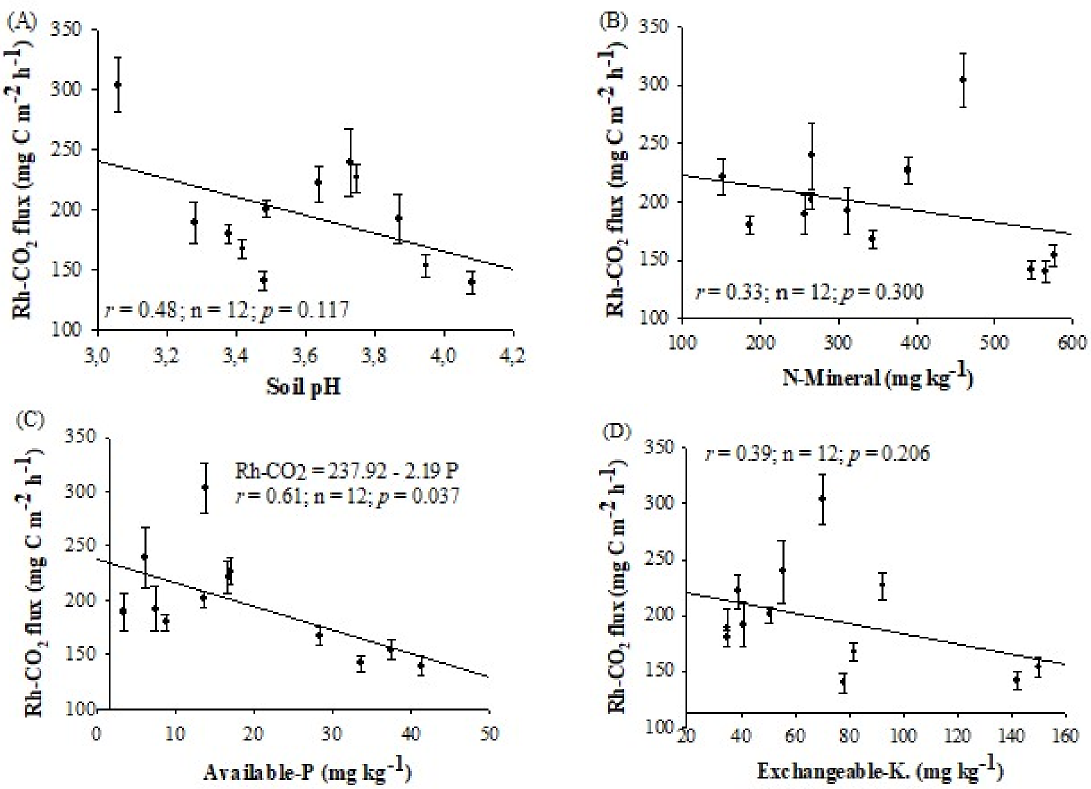

Figure 5. Relationship between Rh-CO_2 flux with (A) Soil pH; (B) N-Mineral; (C) Available-P; (D) Exchangeable-K.

Root exudates contain released ions (i.e. H^+), inorganic acids, oxygen, and water, but mainly consist of carbon-based compounds (Bais et al., 2006). Root exudate is a key factor in nutrient mineralization and an important mediator for soil microbes-plant interaction (Pierret et al., 2007). The solubility of soil-P increases with the increasing organic acids in soil (Jones 1998), and it causes a decrease in soil pH. Soil-K plays as an essential element in activating various enzymes (Szczerba et al., 2009). Besides being produced by roots, enzymes are produced by microbes that are supported by microbial abundance in the rhizosphere. Several researchers stated that respiration of heterotrophic components is influenced by several environmental factors such as soil temperature, groundwater level and soil moisture or water content (Gomez-casanovas et al., 2012; Savage et al., 2013; Wakhid et al., 2017). Soil water content is affected by rainfall. Water plays a role in all intra and extracellular reactions that support microbial life and the movement of solutes. Soil microbial communities are well adapted to a broad range of groundwater environments (Moyano et al., 2013). In our research results, there was no significant relationship between Rh-CO_2 flux and soil temperature, groundwater level, and soil water content observed during the observation period (Figure 6). The dynamics of groundwater level affect the CO_2 flux. Peat decomposition is a complex process because it involves microbial activities. Increased microbial activity occurs when C-substrate and nutrients are available and supported by the environmental conditions (such as soil temperature and soil moisture) suitable for their activity (Kuzyakov and Gavrichkova, 2010; Moyano et al., 2013).

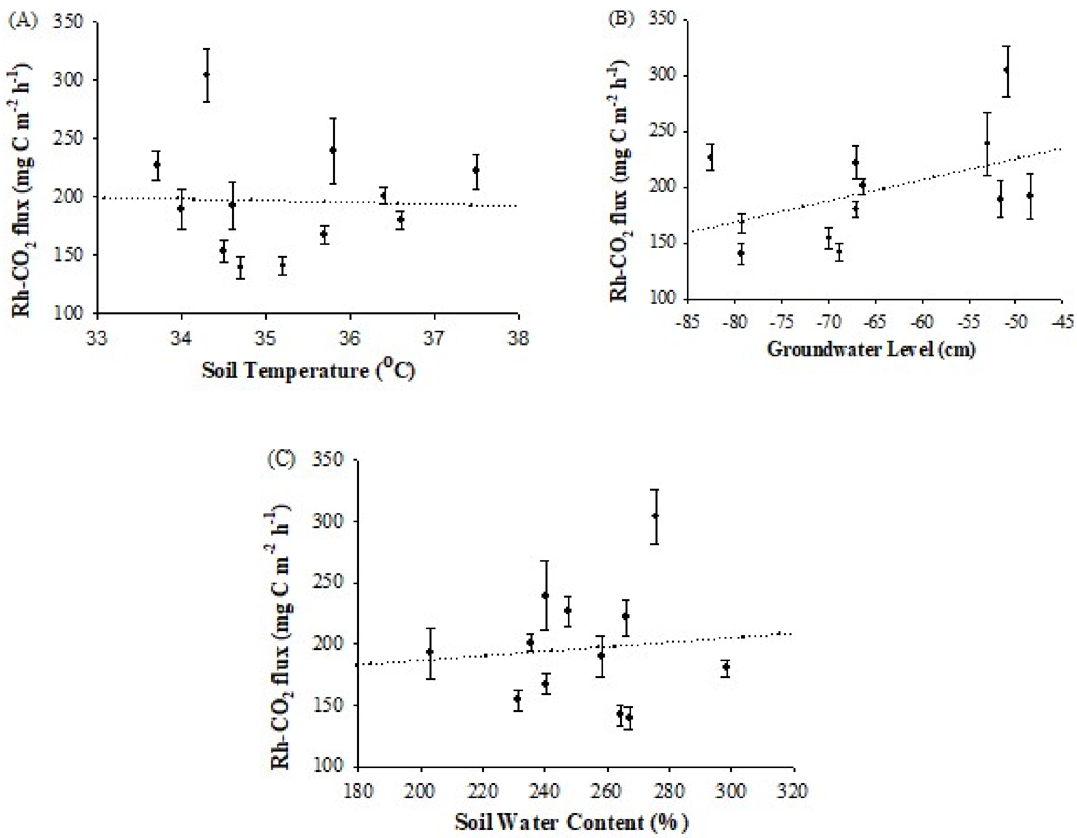

Figure 6. Relationship between Rh-CO_2 flux with: (A) Soil temperature; (B) Groundwater level, and (C) Soil water content. The dotted line represents a non-significant relationship.

Several studies have reported inconsistency of the relationship between CO_2 flux and soil moisture, and these were caused by the complex mechanism of CO_2 production, particularly those involving microbes (Taneva and Gonzalez-Meler, 2011), and the difficulty in separating the effects of osmotic stress, solute diffusion, aeration and its relationship with humidity (Moyano et al., 2013). Tang and Baldocchi (2005) and Wu et al. (2011) stated that soil temperature and soil moisture indirectly affected the decomposition of soil organic matter contributed to CO_2 flux, although the effect had not been seen in this study yet. The variation of soil temperature is influenced by sunlight. A slight variation of soil temperature in the tropics is caused by the optimal use of sunlight for photosynthesis. Based on meta-analysis (Wu et al., 2011), soil respiration was more sensitive to the changes in rainfall than temperature. In this study, monthly rainfall showed a positive correlation with CO_2 flux (Figure 7). Rainfall can affect CO_2 flux indirectly through the movement of root exudates into the environment. Regarding the discussion above, soil properties and environmental factors have a distinct role in the respiration of heterotrophic components. The main controlling factors of Rh-CO_2 flux could be determined by performing multiple regression analysis incorporating several soil determinants (pH, C-organic acids, N-NH_4, N-NO_3, available-P, and exchangeable-K) and environmental factors (soil temperature, soil water content, groundwater level and rainfall) as the independent variables. The results of the analysis showed that there were multiple co-linearity among these variables so that not all the soil and environment parameters were included in the mathematical equation.

The resulted multiple regression equations were only incorporating soil pH, C-organic acids, N-mineral, available-P, and exchangeable-K and soil water content and groundwater level. The regression equation is written below:

$$\text{Rh-CO}_2 \text{ Flux} = -95 + 46.4 \text{ C-organic acids} + 43.5 \text{ soil pH} - 0.368 \text{ soil water content} + 0.087 \text{ N-Mineral} - 5.46 \text{ available-P} + 0.141 \text{ exchangeable-K} - 3.46 \text{ groundwater level}$$

In this equation, N-mineral (the sum of N-NH_4 and N-NO_3) was used as a determinant due to the collinearity between N-NH_4 and available-P. Available-P showed a negative correlation to Rh-CO_2 flux (p = 0.037). The equation showed that the C-organic acids was a significant determinant (p = 0.039) and positively correlated (p = 0.018) to the Rh-CO_2 flux with the coefficient of determination (R^2) of 90.71%.

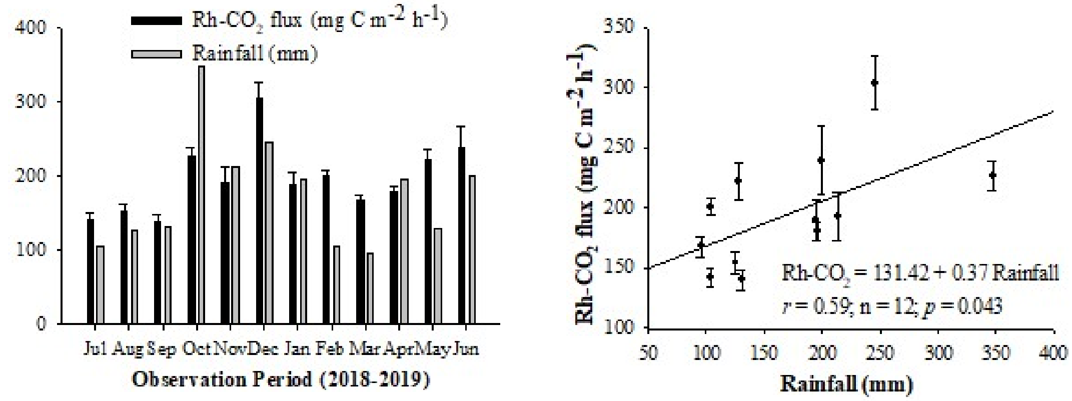

Figure 7. Regression and correlation between Rh-CO_2 flux with rainfall.

This finding emphasizes the importance of substrate in microbial decomposition. Related to the climate change issue, it is important to understand that it is not always the peat that decomposed but the substrate. It is generally assumed that decomposition causes peat loss and peat subsidence. This assumption is not correct. Peat subsidence can happen due to compaction and consolidation of the peat (Melling et al., 2013b). Peat compaction can be seen from the increase in bulk density (BD), for example, from 0.05 g cm^{-3} to 0.1 g cm^{-3}. Increasing BD causes peat maturity to increase and decrease the peat thickness. In addition, the coarse-grained peat material becomes fine-grained peat one, and finally, peat compaction occurs due to shrinkage of the peat pore. In this case, the peat is not lost but compacted. In contrast to peat material dissolution, although the process will dissolve carbon into the aquatic environment in the form of DOC, the dissolved carbon is still smaller than the peat volume. One of the activities that can trigger the dissolution of peat is fertilization. Therefore, fertilizer application on peat soil must be managed carefully and wisely.

Conclusion

Substrate availability as C-organic acids was the main limiting factor and the main determinant of CO_2 flux from heterotrophic respiration components in tropical peatlands. The substrate availability in bulk soil was limited as well as the nutrients so that the possibility of substrate decomposition by microbes was low with an implication of the low release of CO_2 to the atmosphere. In tropical peatland, the lower groundwater level is not the cause of oxidation of the peat having poor nutrient content. The decomposition of peat is mostly influenced by microbial activities.

Acknowledgements

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