Cacao leaf litter decomposition under different moisture and pH: Characteristic of soil N mineralization ($\text{NH}_4^+$ and $\text{NO}_3^-$) and Greenhouse gas $\text{CO}_2$, $\text{CH}_4$, $\text{N}_2\text{O}$ flux emission

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Abstract. Plants litter as soil organic matter turn over to soil by the decomposition process. Decomposition is regulated by environmental factors, such as soil water content and pH. High water content (90%) and pH 6.2 increased $\text{CO}_2$ gas production rate, lowest water content (30%) and pH 4.9 increased $\text{NH}_4^+$ N-mineralization into the soil as decomposition result. In this study, we investigated cacao leaf litter decomposition in soil. Cacao leaf litter was applied in a 100 mL polypropylene bottle with 70 g air-dried soil, but only the soil was applied as a control. For the litter-amended treatments, 2 g (oven-dry weight) with 2 mm sized cacao leaf litter was placed on the soil surface. The treatment with different soil moisture and pH regimes were soil moisture and pH of 30% and 4.9 (SLWC30i), 30% and 6.2 (SLWC306), 60% and 4.9 (SLWC60i), 60% and 6.2 (SLWC606), 90% and 4.9 (SLWC90i), 90% and 6.2 (SLWC906), respectively. All treatment was incubated at 25°C under the dark condition for 28 days. Extractable $\text{NO}_3^-$ and $\text{NH}_4^+$ concentration, and soil greenhouse gas ($\text{CO}_2$, $\text{CH}_4$, $\text{N}_2\text{O}$) were measured. Highest extractable $\text{NO}_3^-$ and $\text{NH}_4^+$ concentration showed in SWC906 (30.7 mg N kg$^{-1}$) and SLWC606 (87.1 mg N kg$^{-1}$), respectively. Soil greenhouse gas production rate highest in water content 90% pH 6.2, $\text{CO}_2$ was 3.82 kg C period$^{-1}$, $\text{CH}_4$ was 0.0089 kg C period$^{-1}$, $\text{N}_2\text{O}$ was 0.016 kg N period$^{-1}$.

1. Introduction
Litter decomposition is the process that energy and nutrients fixed during photosynthesis are released into the atmosphere and soil. Although litter decomposition has been studied by focusing on its decomposition, little consideration for the ultimate fate of the mass once has been paid [1].

The production of carbon dioxide ($\text{CO}_2$) organisms and the plant parts of soil defined in soil respiration. Soil microorganisms gain energy by catabolism of organic matter and emit $\text{CO}_2$ by respiration of plant and microorganisms, in contrast with the aboveground respiration of plant. $\text{CO}_2$ production via litter decomposition in the litter layers is generally included in soil respiration (or belowground respiration) [2].

Technically, the rate of $\text{CO}_2$ production in the soil (i.e., the soil respiration rate) cannot be measured directly in the field. Measurement often made at the soil surface to quantity a rate of $\text{CO}_2$ efflux from the soil to the atmosphere. At a steady-state, the $\text{CO}_2$ efflux rate at the soil surface equals
the rate of CO$_2$ production in soil. In this case, soil CO$_2$ efflux is practically equivalent to soil respiration, and two terms are thus interchangeable [2].

Here the interaction of cacao litter decomposition under different water content and pH was assessed. The aims of this study are to calculate N-mineralization and greenhouse gas, CO$_2$, CH$_4$, N$_2$O emissions induced by cacao litter decomposition under different water content and pH.

2. Methodology
Cacao leaf litter was collected in cacao plantation (120°48ʹ26ʺE longitude s/d 02°31ʹ53ʺ S latitude) located at Tomoni, Bringin Jaya Village, East Luwu South Sulawesi, Indonesia. Cacao leaf litter was randomly collected based on the methodology by [3] in the cacao plantation in which the main 0.25 m$^2$ in a plot of 50 cm x 20 cm) were established for determining litter biomass. Cacao litter was collected from surface soil in early March 2018. The weight of the whole litter was measured and approximately 200 g of the samples were collected as subsamples [4]. Cacao litter with symptoms of obvious herbivory was avoided. Cacao litter samples collected from ten plots randomly placed in the same plot were mixed, air-dried by oven at 70 °C to the constant weight, milled to pass through a 2 mm sieve [5]. Soils used for incubation were collected in University farm, Ehime University, Japan. Soils collected at 0-20 cm depth. In the laboratory, the soil was homogenized air-dried, sieved through a 2 mm mesh, and removed larger organic fragments such as root and litter by hand.

The soil was managed based on a different level of soil moisture content and pH. Soil water content and pH in treatments were 30% and 4.9 (SLWC30i), 30% and 6.2 (SLWC306), 60% and 4.9 (SLWC60i), 60% and 6.2 (SLWC606), 90% and 4.9 (SLWC90i), 90% and 6.2 (SLWC906), respectively. Treatment without cacao litter was also set up with different soil moisture content and soil pH, 30% and 4.9 (SWC30i), 30% and 6.2 (SWC306), 60% and 4.9 (SWC60i), 60% and 6.2 (SWC606), 90% and 4.9 (SWC90i), 90% and 6.2 (SWC906), respectively. The soil was taken in a polypropylene bottle (100 mL0 with 70 g of dry weight. In the litter-amended treatments, 2 g of dry weight cacao litter was placed on the soil surface. Soil water content and moisture were maintained every 2 days. All experimental units will be incubated at 25°C under the dark conditions for 28 days.

Soil samples were analyzed for pH (1:2.5 of soil:and water). Soil moisture content, NH$_4^+$ and NO$_3^-$ concentration (10% KCl extraction) were measured. The NH$_4^+$ concentration was measured using the indophenol blue absorbance method and NO$_3^-$ concentration was measured by colorimetric (Vanadium Chloride) method. N-mineralization rate is estimated by the difference in final concentrations of NH$_4^+$ or NO$_3^-$ [6]. Greenhouse gases, CO$_2$, CH$_4$, N$_2$O were withdrawn from the sample collector by syringe into the evacuated vial bottles. Greenhouse gases were analyzed using gas chromatography (GC-14A and GC-8A) equipped with TCD for CO$_2$, FID for CH$_4$, and ECD for N$_2$O.

\[ F = \rho \times \Delta c / t \times V/S \times T/(T+273) \]

Where F is gases flux (mg m$^{-2}$ hr$^{-1}$), \( \rho \) is gas density (mg m$^{-3}$), \( \Delta c \) is gas concentration (ppmv), t is time interval (hour), V is volume (m$^3$), S is surface area (m$^2$), and T is temperature (K). Cumulative greenhouse gas emission was calculated by the following equation.

\[ Emission = \sum_{n=1}^{j} \{(N_n + N_{n-1}) \times (n - n - 1)/2 \} \]

Where \( N_n \) is the gases flux at the \( n \) sampling \( n-1 \) the day of the \( n \) sampling. Units are converted appropriately according to each survey scale. Production of greenhouse gas was estimated by the difference in the final and initial gas concentration.
3. Result and Discussion

3.1 NO$_3^-$ and NH$_4^+$ concentration and rate N-mineralization.

Figure 1(a) showed the variation of NO$_3^-$ concentrations in SLWC30i, SLWC306, SLWC60i, and SLWC606, the NO$_3^-$ concentrations reached the maximum in 14 to 28 day after incubation (DAI) ranging from 35.69 to 87.08 mg N kg$^{-1}$. In figure 1(b), concentrations of NO$_3^-$ in SWC306, SWC60i, and SWC606 reached the maximum in 14 to 28 DAI ranging from 43.58 to 89.27 mg N kg$^{-1}$. In figure (c), concentrations of NH$_4^+$ reached maximum in SLWC906 at 7 DAI ranging from 27.79 to 31.46 mg N kg$^{-1}$. After that, it decreased from 14 to 28 DAI. In SLWC90i, SLWC60i, SLWC30i, NH$_4^+$ concentration reached maximum at 21 DAI. In SLWC90i, NH$_4^+$ concentration ranged from 9.92 to 22.73 mg N kg$^{-1}$, and ranged from 5.14 to 8.50 mg N kg$^{-1}$ in SLWC60i and SLWC30i. In figure (d), the concentration of NH$_4^+$ in SWC906 and SWC90i reached the maximum at 21 DAI ranging from 27.79 to 34.35 mg N kg$^{-1}$. In SWC30i and SWC306, NH$_4^+$ concentration reached the maximum in 7 DAI ranging from 5.60 to 13.09 mg N kg$^{-1}$. In SWC60i, NH$_4^+$ concentration reached the maximum in 21 DAI ranging from 6.72 to 14.39 mg N kg$^{-1}$.

![Figure 1](image1.png)

![Figure 1](image2.png)

![Figure 1](image3.png)

![Figure 1](image4.png)

Figure 1. Variation of NO$_3^-$ and NH$_4^+$ concentrations.

| Treatment  | NO$_3^-$ concentration (mg N kg$^{-1}$) | Average | SE | NH$_4^+$ concentration (mg N kg$^{-1}$) | Average | SE |
|------------|----------------------------------------|---------|----|----------------------------------------|---------|----|
| SLWC30i    | 55.75 b                                 | 1.84    |    | 5.29 cde                               | 0.24    |    |
| SWC30i     | 56.92 ab                                | 1.99    |    | 2.94 de                                | 0.14    |    |
| SLWC306    | 70.85 ab                                | 4.1     |    | 2.83 de                                | 0.44    |    |
| SWC306     | 71.75 ab                                | 3.32    |    | 2.33 e                                 | 0.38    |    |
| SLWC60i    | 83.47 a                                 | 3.23    |    | 4.23 cde                               | 0.48    |    |
| SWC60i     | 83.96 a                                 | 4.52    |    | 3.47 de                                | 0.54    |    |
| SLWC606    | 87.07 a                                 | 9.46    |    | 2.86 de                                | 0.13    |    |
| SWC606     | 89.27 a                                 | 9.7     |    | 2.57 de                                | 0.43    |    |
| SLWC90i    | 21.55 c                                 | 14.5    |    | 12.93 bc                               | 7.07    |    |
| SWC90i     | 21.53 c                                 | 14.48   |    | 17.76 b                                | 5.35    |    |
| SLWC906    | 12.75 c                                 | 1.5     |    | 11.23 bcd                              | 8.35    |    |

Table 1. Analysis of variance in NO$_3^-$ and NH$_4^+$ concentrations.
Table 1 showed that significantly different in NH$_4^+$ and NO$_3^-$ concentrations among the treatment were observed. A similar result was found in the previous study [7]. Table 1 also showed that the highest NO$_3^-$ concentrations were observed at 60% and 30% of moisture contents. A similar result was also reported [8]. Increasing soil moisture content resulted in lower NO$_3^-$ concentration which indicated nitrification decreased with increasing soil moisture, which was also observed in the previous study [9-11].

Production rates of NO$_3^-$ and NH$_4^+$ were shown in figure 2. Under the pH 4.9 and moisture 90%, NO$_3^-$ production rate showed the highest of 0.02 mg N kg$^{-1}$ period$^{-1}$. The production rate of NH$_4^+$ under the pH 4.9 and moisture content of 30% and pH 4.9 and moisture content of 60% were 2.34 and 0.76 mg N kg$^{-1}$, respectively. Under the pH 6.2 and moisture content of 30%, the production rate of NH$_4^+$ (0.49 mg N kg$^{-1}$) was higher compared to under the pH 6.2 and moisture content of 60% (0.29 mg N kg$^{-1}$). The highest production rates of NO$_3^-$ and NH$_4^+$ were observed under the pH 4.9 and moisture content of 90% and pH 4.9 and moisture content of 30%, respectively.

![Figure 2. NO$_3^-$ (a) and NH$_4^+$ (b) production rates under moisture content of 30%, 60%, 90% and pH 4.9 and pH 6.2.](image)

### 3.2 Greenhouse gas fluxes

Variations of greenhouse gas fluxes were shown in figure 3. CO$_2$ flux treatment with cacao leaf litter reached the maximum in 7 DAI in all treatment, then, decreased from 21 to 28 DAI. The highest and lowest CO$_2$ fluxes in the treatment with cacao litter were observed in SLWC60i (1315.03 mg C g$^{-1}$ hr$^{-1}$) and in SLWC606 (835.63 mg C g$^{-1}$ hr$^{-1}$), respectively. CO$_2$ flux treatment without cacao litter showed the maximum in 7 DAI. The highest and lowest CO$_2$ fluxes in the treatment without cacao litter were observed in SWC906 (109.92 mg C g$^{-1}$ hr$^{-1}$) and in SWC30i (276.31 mg C g$^{-1}$ hr$^{-1}$), respectively. CH$_4$ flux treatment with cacao litter reached a maximum of 14 DAI. The highest CH$_4$ flux treatment with cacao litter was observed in SLWC906 (3.96 mg C g$^{-1}$ hr$^{-1}$). CH$_4$ flux treatment without cacao litter reached the maximum in 14 DAI. The highest CH$_4$ flux treatment without cacao litter was observed in SWC90i (0.28 mg C g$^{-1}$ hr$^{-1}$). N$_2$O flux reached the maximum in 21 DAI in treatment of SLWC906 (6.45 mg N g$^{-1}$ hr$^{-1}$) and in 28 DAI in SLWC90i (6.24 mg N g$^{-1}$ hr$^{-1}$). The highest N$_2$O flux was observed in SWC906 (1.29 mg N g$^{-1}$ hr$^{-1}$) in 14 DAI.
Figure 3. Variation of CO₂ (a, b), CH₄ (c, d), and N₂O (e, f) fluxes.

Table 2. Analysis of variance relationship treatment and Variation flux gas CO₂, CH₄, N₂O

| Treatment | CO₂ (mg C g⁻¹ hr⁻¹) Mean | SE | CH₄ (mg C g⁻¹ hr⁻¹) Mean | SE | N₂O (mg N g⁻¹ hr⁻¹) Mean | SE |
|-----------|----------------------------|----|---------------------------|----|---------------------------|----|
| SLWC30i   | 1050.16 a                  | 298.22 | -0.02 a                  | 0.07 | 0.11 b                  | 0.05 |
| SWC30i    | 46.42ba                    | 132.17 | -0.03 a                  | 0.11 | 0.03 b                  | 0.04 |
| SLWC306   | 844.39 ab                  | 496.06 | 0.29 a                   | 0.64 | 0.14 b                  | 0.28 |
| SWC306    | -55.20 b                   | 94.55  | -0.06 a                  | 0.08 | -0.01 b                 | 0.26 |
| SLWC60i   | 1488.09 a                  | 154.55 | -0.23 a                  | 0.26 | 0.06 b                  | 0.13 |
| SWC60i    | 209.53 b                   | 118.22 | 0.02 a                   | 0.08 | 0.03 b                  | 0.04 |
| SLWC606   | 835.63 ab                  | 866.23 | 0.03 a                   | 0.18 | 0.07 b                  | 0.04 |
| SWC606    | 124.21 b                   | 94.55  | 0.15 a                   | 0.3  | 0.19 b                  | 0.26 |
| SLWC90i   | 929.74 ab                  | 1165.3 | 0.15 a                   | 1.49 | 6.24 a                  | 7   |
| SWC90i    | 151.81 b                   | 46.78  | 0.54 a                   | 0.91 | 0.28 b                  | 0.33 |
| SLWC906   | 1563.36 a                  | 328.45 | 0.50 a                   | 0.51 | 4.79 ab                 | 2.93 |
| SWC906    | 191.96                     | 115.27 | 73.99 a                  | 218.26 | 0.01 b                  | 0.04 |

Different letters represent significant differences (P < 0.05).
3.3 Greenhouse gas production.

In figure 4 (a), the highest CO$_2$ production rate was 3.82 kg C period$^{-1}$ in pH 6.2 and moisture content of 90%. The highest CH$_4$ production rate was 0.0089 kg C period$^{-1}$ in pH 6.2 and moisture content if 90%. The highest N$_2$O production rate was 0.016 kg N period$^{-1}$ in pH 6.2 and moisture content of 90%.

![Figure 4](image-url)

(a) 
(b) 
(c)

**Figure 4.** Greenhouse gas production rate under different conditions of pH (4.9 and 6.2) and moisture content (30%, 60%, 90%).

Figure 3 and 4 in line with table 2 showed that variation flux gas CO$_2$, CH$_4$, N$_2$O is caused due to the interaction between moisture and temperature [12]. Significant differences gas CO$_2$ flux treatment with cacao leaf litter indicated cacao litter decomposition results from the fact that litter decomposition involves three processes: the leaching, fragmentation, and chemical alteration of dead organic matter to produce gas CO$_2$ [2].

4. Conclusion

Decomposition of cacao litter under pH 4.9 and moisture content of 90% showed a high NO$_3^-$ production rate of 2.34 mg N kg$^{-1}$. The highest NH$_4^+$ production rate was observed under pH 6.2 and moisture content of 90%. Highest NO$_3^-$ and NH$_4^+$ concentrations were observed in SWC606 (89.27 mg N kg$^{-1}$) and in SLWC906 (31.46 mg N kg$^{-1}$), respectively. Greenhouse gas emission induced by cacao litter decomposition was highest under condition pH 6.2 with the moisture content of 90%. The result of this study showed that there was a significant difference in CO$_2$ flux among the treatment. The CO$_2$ production rate was highest under the condition of pH 6.2 and moisture content of 90% (3.82 kg C period$^{-1}$).
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