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Non-microbial methane emissions from tropical rainforest soils under different conditions

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Abstract

Non-microbial methane ($NM\text{-}CH_4$), emissions from soil might play a significant role in carbon cycling and global climate change. However, the production mechanisms and emission potential of soil $NM\text{-}CH_4$ from tropical rainforest remain highly uncertain. In order to explore the laws and characteristics of $NM\text{-}CH_4$ emission from tropical rainforest soils. Incubation experiments at different environmental conditions (temperatures, soil water contents, hydrogen peroxide) and for soils with different soil organic carbon (SOC) contents were conducted to investigate the $NM\text{-}CH_4$ emission characteristics and its influence factors of soils (0-10cm) that collected from a tropical rainforest in Hainan, China. Incubation results illustrated that soil $NM\text{-}CH_4$ release showed a linear increase with the incubation time in the first 24 hours at 70 ˚C, whereas the logarithmic curve increase was found in 192 h incubation. Soil $NM\text{-}CH_4$ emission rates under aerobic condition were significantly higher than that of under anaerobic condition at first 24 h incubation. The increasing of temperature, suitable soil water contents (0–100%), and hydrogen peroxide significantly promoted soil $NM\text{-}CH_4$ emission rates at the first 24 h incubation. However, excessive soil water contents (200%) inhibited soil $NM\text{-}CH_4$ emissions. According to the curve simulated from the $NM\text{-}CH_4$ emission rates and incubation time at 70 ˚C of aerobic condition, soil would no longer release $NM\text{-}CH_4$ after 229 h incubation. The $NM\text{-}CH_4$ emissions were positively corelated with SOC contents, and the average soil $NM\text{-}CH_4$ emission potential was about 6.91 ug per gram organic carbon in the tropical mountain rainforest. This study revealed that soils in the tropical rainforest could produce $NM\text{-}CH_4$ under certain environment conditions and it supported production mechanisms of thermal degradation and reactive oxygen species oxidation. Those results could provide a basic data for understanding the soil $NM\text{-}CH_4$ production mechanisms and its potential in the tropical rainforest.

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Introduction

Methane (CH_4), an important greenhouse gas, which contribution to the greenhouse effect is second only to CO_2, and has a major impact on atmospheric chemistry and climate [1]. The concentration of CH_4 in the atmosphere has been increasing since the industrial revolution. It is expected that by the 2030s, the amounts of CH_4 released by human activities will increase by about 25% [2], which may have a major impact on climate change in the future. Nearly 15 to 30 percent of CH_4 in the atmosphere come from soils each year [3]. Studies focus on CH_4 emission mechanisms and potential from soil are critical in understanding carbon cycling and global climate change projections.

Generally, there are two production mechanisms of soils for atmospheric CH_4, one is microbial CH_4 and the other is non-microbial methane (NM-CH_4) [4]. Soil microbial CH_4, produced by the methanogenesis of methanogen, which was considered to be the mainly sources of soil CH_4 emissions. Hao et al. [5] first observed that CH_4 could be released from the soil in the savanna grassland during the dry season, and the CH_4 emission was also detected in the forest soil. Subsequently, Andersen et al. [6] and Fischer and Hedin [7] found soil CH_4 emission in aerobic soils. There are many explanations for the possible underlying mechanisms for this phenomenon, which are based on the microbial perspectives. Rimbault et al. [8] reported that the soil aerobic bacteria which could produce a small amount of CH_4 under certain conditions, while Peter and Conrad [9] reported that the existence of soil anaerobic microhabitats can explain this phenomenon.

Compared with microbial CH_4, soil NM-CH_4 has received less attention in the past decades. The production of soil NM-CH_4 is more extensive than we originally thought in the soil environment. Soil NM-CH_4 emission is an instantaneous reaction product of organic compounds under environmental pressures, which was caused by the cutting off the methyl functional groups of organic compounds [10]. The sources of NM-CH_4 mainly include energy utilization [11], biomass combustion [12], and geological release [13]. In recent years, it has been discovered that plants [14], animals [15], and marine surface water [16] can also produce NM-CH_4 under high temperature [17,18], strong ultraviolet radiation [19] and rich reactive oxygen [20]. Since plants were proved to release NM-CH_4, some research scholars wonder whether soil could also release NM-CH_4 [21]. This new perspective was first demonstrated by Kammann et al. [22], who found that soil samples can still release large amounts of CH_4 even after homogenization (the anaerobic habitats in oil have been destroyed). Then, several recent studies have also confirmed that soil can produce NM-CH_4 under aerobic conditions [10,23–25].

Although some researches on soil NM-CH_4 emissions and its influencing factors were reported in recent years [22–25], the production mechanisms and emission potential of the NM-CH_4 from tropical rainforest soil remain highly uncertain because of limited study. Tropical rainforests, as one of the most important components of forest, are of great importance for the global carbon cycle. To study NM-CH_4 emissions of tropical rainforest soils is of great importance in understanding the carbon cycle of forest ecosystems and CH_4 emission reducing. The Jianfengling Long-term Research Station of Tropical Forest Ecosystem is an unique platform to address the release mechanisms and potential of soil NM-CH_4 emissions. Soil samples at depth of 0–10 cm were collected from 6 plots (20 m × 20 m) in Jianfengling Long-term Research Station of Tropical Forest Ecosystem, and the NM-CH_4 emission rates from soil incubation experiments were observed under four different incubation conditions. The aims of this study are to understand the possible releasing mechanisms and potential of soil NM-CH_4 emissions in tropical rainforest. In this study we hypothesized that NM-CH_4 releases rates would decrease with incubation time, and the soil NM-CH_4 could be influenced by

different temperature, water content condition, hydrogen peroxide (H_2O_2) and soil organic carbon contents.

Materials and methods

Study area

The study area located in Jianfengling National Nature Reserve (18.33˚~18.95˚N, 108.48˚~109.20˚E, altitude range: 0-1412m), south-west Hainan Island, China. This area is characterized by a tropical rainforest climate with a mean annual precipitation of approximately 2400 mm (of which 80–90% falls in May–October) and a mean annual temperature of 24.5 ˚C [26]. The most common soil type is the montane lateritic red or yellow earth [26]. In addition, the study area is highly habitat heterogeneity with rich species composition and complex structure, the dominant species are *Lauraceae*, *Fagaceae*, and *Rubiaceae*. Moreover, the average canopy height in this area is 28.0 m, the density of trees with DBH (diameter at breast height) above 5 cm and 10 cm could reach 170 species and 150 species per hectare, respectively [27,28].

Soil sampling

Total six plots with each of 3 equal-sized subplots (20 × 20 m = 400 m^2) set in, were selected in the Jianfengling Long-term Research Station of Tropical Forest Ecosystem (each location is approved by National Park Administration of Hainan Tropical Rainforest) (Table 1). On April 20, 2019, soil samples at the depth of 0–10 cm were randomly collected from the subplot. In each subplot, five soil cores of 10 cm diameter from the middle to the four corners were mixed to form a composite sample. 18 soil samples were collected in the 6 sites. We also collected two samples with the same sampling method in JFL1 site, and total twenty soil samples were collected. The soil samples were packed in bags in a constant cool temperature, and then transported into the laboratory and stored at 4 ˚C in a refrigerator. After a week of soil stabilization, soils were passed through a 2 mm diameter mesh and divided into two parts. The first part was air-dried, homogenized, and used for the analysis of soil properties and the other was stored in the 4 ˚C for the incubation experiment.

In addition, in order to study the relationships between SOC (soil organic carbon) and NM-CH_4 emissions, the other twenty soil samples were collected from tropical rubber forest (RF1) in June, 2019 (Table 1).

Soil chemical analysis

SOC was determined by concentrated sulfuric acid-potassium dichromate oxidation method [29]. Soil total nitrogen (TN) and soil total phosphorus (TP) were extracted by semimicro kelvin method and determined by automatic flow analyzer (PROXIMA 1022/1/1, ALLIANCE

Table 1. Geographical information of soil sampling sites in the tropical rainforest.

Sites	Latitude (N)	Longitude (E)	Altitude (m)	Slope (˚)	Aspect
JFL1	18˚43′54.74″	108˚53′10.04″	872	2.9	north
JFL2	18˚43′53.23″	108˚53′17.13″	855	10.8	northeast
JFL3	18˚43′53.20″	108˚53′22.90″	884	7.8	north
JFL4	18˚43′55.71″	108˚53′30.93″	810	2.0	northeast
JFL5	18˚43′54.05″	108˚53′30.23″	892	7.6	north
JFL6	18˚43′59.70″	108˚53′39.10″	830	4.5	southwest
RF1	19˚01′12.01″	109˚58'11.99"	127	1.0	-

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Table 2. Descriptive statistics of the soil properties in the tropical rainforest of Jianfengling National Nature Reserve.

Variable	Max	Min	Average	STDEV[e]	CV[f]
SOC[a] (g/kg)	42.44	31.23	36.18	5.59	0.151
TN[b] (g/kg)	1.66	0.83	1.33	0.29	0.066
TP[c] (g/kg)	0.14	0.06	0.10	0.03	0.050
SWC[d] (%)	23.00	13.85	18.75	3.18	0.025
pH value	6.10	5.11	5.46	0.26	0.015

[a] soil organic carbon.

[b] Soil total nitrogen.

[c] soil total phosphorus.

[d] soil water content.

[e] standard deviation.

[f] coefficient of variation.

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instruments, France) [30]. Soil water content (SWC) was determined by drying method. Soil pH value was determined by potentiometric method (the soil water ratio = 1:2.5), the basic physical and chemical indexes of soil were showed in Table 2.

Incubation experiment

Autoclaving is the most widely used sterilization method [10]. We firstly sterilized the soil with high-pressure steam (30 min, 121 ˚C) to eliminate the influence of microbial CH_4, then freeze-drying and homogenization the soil. Finally, about 10 g of soils were transferred to a 100 mL serum bottle, which was then sealed with a high temperature resistant butyl rubber stopper.

Before others experiments, the soil NM-CH_4 emission characteristics at first 192 h (air samples collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 48, 72, 96, 120, 144, 168 and 192 h respectively) were studied from the incubation experiment in aerobic and anaerobic environment at 70 ˚C with natural soil water contents. The incubation treatments are as follows, different temperature, various soil water contents, hydrogen peroxide, and SOC contents.

1. To study effects of different temperature on NM-CH_4 emissions, the incubation experiments with natural soil water contents were conducted at 30 ˚C, 40 ˚C, 50 ˚C, 60 ˚C and 70 ˚C. The different incubation temperatures were achieved through incubators, and the anaerobic environment was created by blowing high-purity nitrogen [31].
2. According to the natural water contents of the soil samples, ultrapure water or freeze-drying were applied to adjust the soil water content into 8 gradients, which are 0%, 5%, 10%, 30%, 50%, 70%, 100%, and 200%, and the incubation was in aerobic environment at 70 ˚C for 24 hours. Because the incubation experiments with different temperatures showed that 70 ˚C was most beneficial to soil NM-CH_4 emissions.
3. The mass concentration of H_2O_2 concentrations were setting at 0%, 0.1%, 0.25%, 0.5%, 1%, and 2%. The incubation experiment was conducted in aerobic environment at 30 ˚C (with natural soil water contents) for 24 hours. Because the incubation experiments with different temperatures showed that the NM-CH_4 emissions at 30 ˚C are weak, which can avoid the influences of temperature on NM-CH_4 emissions.
4. Besides the twenty soil samples from the Jianfenling tropical rainforest, the other 20 soil samples were collected from the tropical rubber forest (Table 1). The NM-CH_4 emission

characteristics for the forty soil samples were measured by incubation experiment in aerobic environment at 70 ˚C (with natural soil water contents) for 24 hours.

CH_4 concentration measurement

Air samples sampling was performed before and after incubation. Before sampling, syringe was used to blow several times to mix the gas and then took 1 ml gas sample. This operation was carried out to minimize the interference to the sample in the incubation flask. For the continuous observation experiment, an equal volume of compressed air or high-purity nitrogen needs to be injected into the incubation flask right after the extraction of gas sample to maintain the pressure inside the incubation flask.

The CH_4 concentrations in air samples were measured by a gas chromatograph (7890A, Agilent Co., USA) [26]. The gas chromatograph was equipped with a flame ionization detector (FID). The operating temperature was 250 ˚C and oven temperature was maintained at 90 ˚C. The fuel gas was H_2 (40 mL min^{-1}) and the combustion supporting gas was air (400 mL min^{-1}). The standard gas concentration of CH_4 was 2.0 ppmv, provided by Beijing AP-BAIF Gases Industry Co., Ltd.

Data analysis

The emission rates of CH_4 was calculated based on the change of CH_4 concentration inside the incubation flask [14]. The total CH_4 emission fluxes for different SOC contents samples in 229 h incubation were calculated by fluxes in first 24 h divided the proportions of it to total fluxes. One-way ANOVA followed by Tukey's multiple comparison tests was used to establish significant differences of soil NM-CH_4 emission among the temperature gradient, the concentration of H_2O_2 solution, soil water content. The difference at $P<0.05$ level considered to be statistically significant. The statistical analyses were carried out in SPSS 22.0. Figures were generated using the software Origin 2018.

Results

Soil NM-CH_4 emission characteristics as incubation time increasing

The concentration of soil NM-CH_4 emission was measured at different incubation time, and results indicated that the emission flux of soil NM-CH_4 increased with incubation time in the first 129 h (Fig 1). Within 24 h, the NM-CH_4 emission flux showed a significant positive linear correlation with the time change ($P<0.01$ $R^2 = 0.98$). However, within 192 h, the NM-CH_4 emission rate showed a gradual decrease trend. In addition, the relationship between NM-CH_4 emission rate and incubation time was explained by the logarithmic function y = -0.19ln(x-10.19) + 0.11 ($R^2 = 0.97$) (Fig 2), which indicated that the soil would no longer release NM-CH_4 when the incubation time was up to 229 h. The soil NM-CH_4 emission at first 24 h account for 33.14% of total emission amounts in aerobic environment at 70 ˚C with natural soil water contents, which means that substrates related to NM-CH_4 emission during this period are sufficient. Therefore, it is important to study NM-CH_4 emissions within 24 hours.

Influence of temperature on soil NM-CH_4 emission

To study the effect of temperature on soil NM-CH_4, soil samples were incubated at temperatures ranging from 30 to 70 ˚C with natural soil water contents under aerobic and anaerobic conditions, respectively. The results indicated that the CH_4 emission rate gradually increased ($+1.11\times10^{-3}$–0.15 μg/g SOC/h) with increasing of temperature at first 24 h (Fig 3). At lower

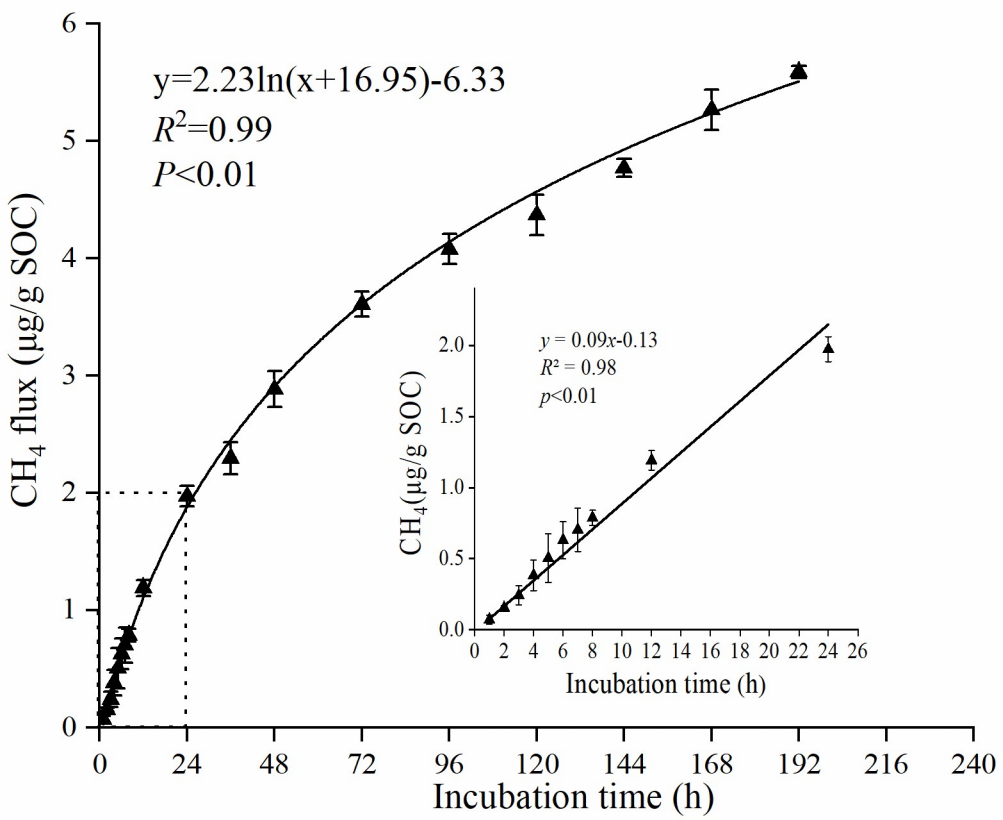

Fig 1. Emission flux of soil NM-CH_4 in aerobic environment at 70 ˚C with natural soil water contents in first 192 h.

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temperatures (≤50 ˚C) conditions, the CH_4 emission rate was less sensitive to temperature change, and no significantly change was observed in the CH_4 emission rate, especially in the anaerobic environment (the emission rate of NM-CH_4 were lower than 0.06 μg/g SOC/h). However, the CH_4 emission rates changed dramatically when the temperature was high (60–70 ˚C) in both aerobic and anaerobic conditions, and the emission rates increased by 1–3 times per 10 ˚C (the emission rates of NM-CH_4 ranged from 0.05 to 0.15 μg/g SOC /h). In addition, there was a significant positive correlation between the emission rates of NM-CH_4 under aerobic and anaerobic conditions (P<0.05), and the emission rates under aerobic condition was always higher than that of under the anaerobic condition in a certain temperature.

Influence of H_2O_2 on soil NM-CH_4 emissions

To study the relationships between soil NM-CH_4 emission and H_2O_2 contents, the soil samples were added with 5 ml H_2O_2 solution with different concentration (0%, 0.1%, 0.25%, 0.5%, 1% and 2%) at 30 ˚C with natural soil water contents. The results revealed that the NM-CH_4 emission rates increased with the increasing of H_2O_2 contents (+0.07–0.77 μg/g SOC/h), and the emission rates showed a significant positive linear correlation with H_2O_2 concentration (P<0.01, R^2 = 0.96) (Fig 4).

Emission characteristics of soil NM-CH_4 at different soil water contents

The emission characteristics of soil NM-CH_4 in tropical rainforest at 70 ˚C were showed in Fig 5. The results of the study indicated that there was a mutation of NM-CH_4 emission rates

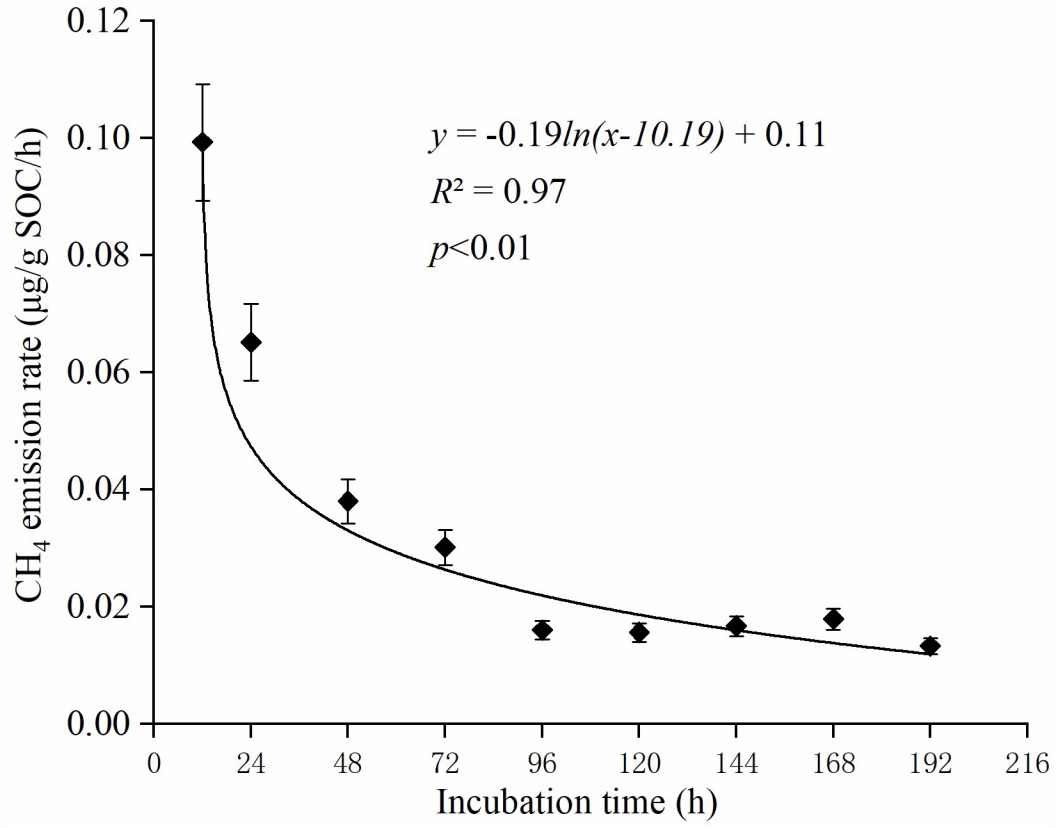

Fig 2. Fitting relationship between NM-CH_4 emission rates and incubation time in aerobic environment at 70 ˚C with natural soil water contents in first 192 h.

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when soil water contents increased from 0% to 5%. The results also showed that soil water contents increasing promoted NM-CH_4 emissions (+0.02–0.31 μg/g SOC/h), and the effect decreased significantly at first and then decreased gradually. In addition, the CH_4 emission rate reached the peak at the soil water content of 5% (0.42 μg/g SOC/h). When the soil water content was 200%, the emission rate was 0.05μg/g SOC/h, which was lower than that of at soil water content of 0% (0.07 μg/g SOC/h). The results indicated that the excessive soil water contents inhibited the NM-CH4 emissions.

Relationships between SOC contents and soil NM-CH_4 emissions

The soil NM-CH_4 emission fluxes were positively correlated with SOC in the both tropical rubber forest ($P<0.01$, $R^2 = 0.81$) and tropical rainforest ($P<0.01$, $R^2 = 0.79$), respectively. In addition, the total soil NM-CH_4 emission fluxes in the tropical rainforest (0.21±0.05 ug/g(dw)) were higher than those of in the tropical rubber forest (0.06±0.02 ug/g(dw)) (Fig 6). The average NM-CH_4 emission fluxes were 6.91 μg per gram organic carbon in the tropical forest of Hainan.

Discussion

Soil NM-CH_4 emission characteristics at different incubation time

There was a linear relationship between soil NM-CH_4 emissions and incubation time at the first 24 h (Fig 1), which was consistent with the results of Hurkuck et al. [24]. Our results also

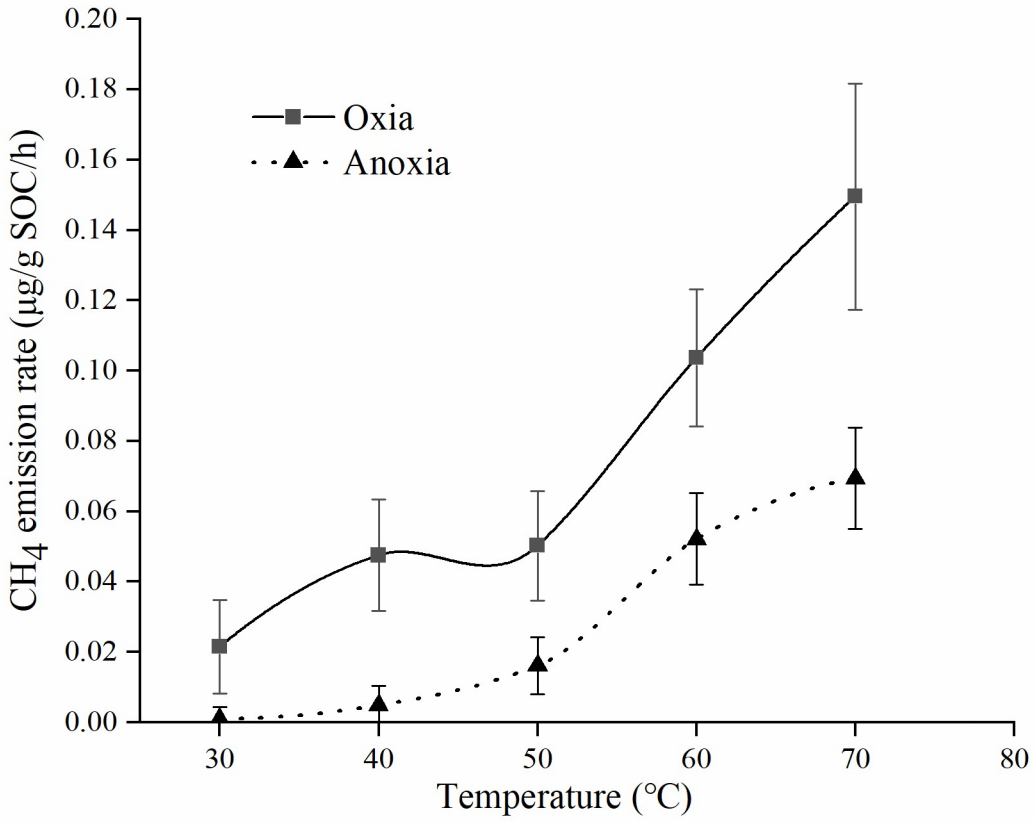

Fig 3. The emission rates of NM-CH_4 at different incubation temperature from 30˚C to 70 ˚C and under aerobic and anaerobic conditions.

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showed that soil NM-CH_4 emission rates gradually decreased as the incubation time increasing from 24 h to 192 h. The NM-CH_4 emission rates were determined by the type and contents of NM-CH_4 precursors in soil [4]. It could be deduced that some of the precursors are consumed as the incubation time increased, resulting in a gradual decrease in the NM-CH_4 emission rates after 24 h in this study. Moreover, it could be concluded that there was no soil NM-CH_4 emission after 229 hours incubation from the logarithmic function (Fig 2), which may due to the contents of NM-CH_4 precursors were nearly exhausted. The emission rates of soil NM-CH_4 at first 24 h were higher in aerobic condition than those of in anaerobic conditions (Fig 3). Wang et al. [10] also found that the NM-CH_4 emission rates under aerobic condition was higher than that under anaerobic condition, and the differences intensified with the increase of temperature. Therefore, the following discussion focused on the influences of different temperature, H_2O_2 concentration, soil water contents and SOC contents on NM-CH_4 emission rates at first 24 h under aerobic incubation.

Higher temperature, H_2O_2 concentration, soil water content promoted soil NM-CH_4 emission

The thermal degradation mechanism as a major mechanism of NM-CH_4 production is gradually accepted [4]. Previous studies reported that there were many functional groups including methyl, methoxy and methyl sulfide (NM-CH_4 precursors) existed in soil and they could produce CH_4 [18,32]. The NM-CH_4 emission rates increased gradually with the increasing of

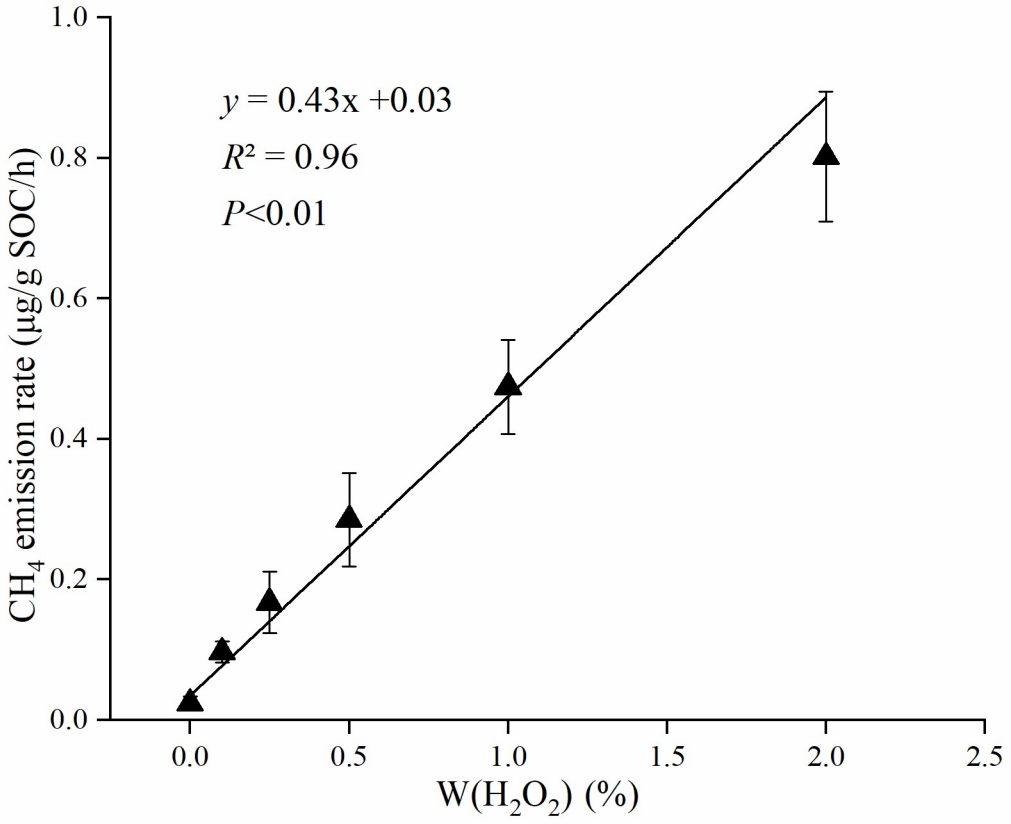

Fig 4. The emission rates of NM-CH_4 at different concentrations of H_2O_2 (0, 0.1%, 0.25%, 0.5%, 1%, 2%) in aerobic environment at 30 ˚C.

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incubation temperature at first 24 h in this study (Fig 3). It indicated that high temperature could accelerate the NM-CH_4 emission, because the higher temperature could dissociate much more soil chemical bonds of NM-CH_4 precursors [10,23,25], and resulting in higher CH_4 production and emission rates.

Reactive oxidative species (ROSs, such as hydroxyl radical) are highly oxidative [33], capable of cleaving functional groups that can produce NM-CH_4. Soil biological activities can exude ROSs and soil itself can also produce ROSs through photochemical reactions with certain mineral oxides [34,35]. The results in this study showed that the soil NM-CH_4 emission rates were positively correlated with the H_2O_2 concentration at the first 24 h (Fig 4). The results of Jugold et al. [25] also found a similar pattern in aerobic soil. However, these results were different from the result of Wang et al. [10] that there was a logarithmic growth relationship between CH_4 emission rates and H_2O_2 concentration. The interesting thing is, some researchers have found the different patterns in plants, for example, Wang et al. [31] found that the plant pectin showed a logarithmic emission characteristic, while the dry leaves showed a linear emission characteristic. Those indicated that the carbon sources may be an important factor affecting CH_4 emission under the different H_2O_2 concentrations environment.

It is important to study the effect of soil water content on the release of NM-CH_4, because soil water content always changed in forest due to precipitation and evaporation. In this study, NM-CH_4 emission rates had a sudden increased as soil water content increasing from 5% to 10%, and it gradually decreased with soil water content increased from 10% to 100% at the

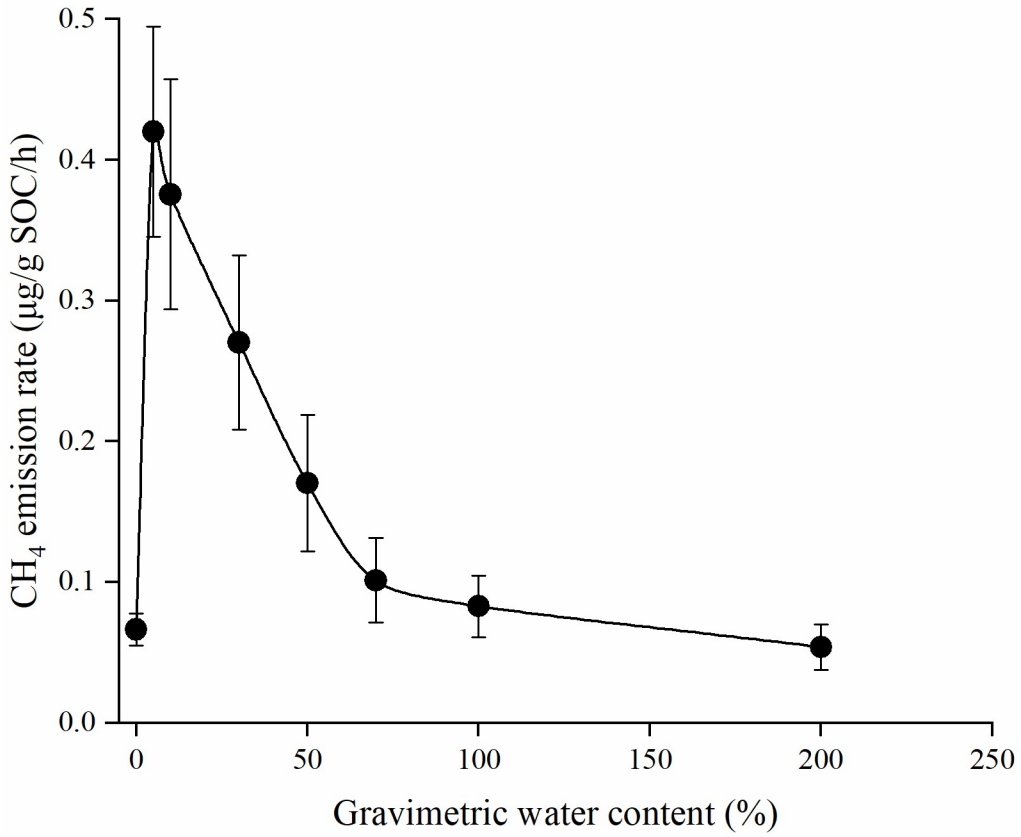

Fig 5. The emission rates of NM-CH_4 at different soil water contents (0%, 5%, 10%, 30%, 50%, 100%, 200%) treatments in aerobic environment at 70 ˚C.

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first 24 h (Fig 5), which indicated that appropriate water can effectively promote NM-CH_4 emissions. However, CH_4 emission rate was decreased when soil waters content reached 200%, indicating the excessive soil water would inhibit NM-CH_4 emissions. The results are consistent with the previous results from Wang et al. [10] and Jugold et al. [25]. Soil water contents could affect NM-CH_4 release from chemical degradation of organic compounds, and it also affects oxygen concentration and the microenvironment where methanogenesis takes place [24]. More research is needed on the mechanisms of soil water contents affecting NM-CH_4 emissions in the future. For example, what is the internal mechanism of moisture affecting soil NM-CH_4 emission, how to explain its internal mechanism at the level of organic chemistry, and where is the critical point of the impact of moisture on soil NM-CH_4 emission?

Potential of NM-CH_4 emission

This study found that the soil NM-CH_4 emission fluxes were significantly positively correlated with SOC content (Fig 6). When conducting experiments on NM-CH_4 emission from aggregates, Wang et al. found that there is a significant positive correlation between the NM-CH_4 emission rate of soil aggregates and the concentration of organic carbon, indicating that the role of the amount of organic carbon can determine soil NM-CH_4 emissions [10]. Similarly, the studies of Gu et al. and Hurkuck et al. also reported that there is a significant positive correlation between the release rate of soil NM-CH_4 and the soil organic carbon content [23,24]. It supported that soil NM-CH_4 was derived from soil organic matter. But what needs to be

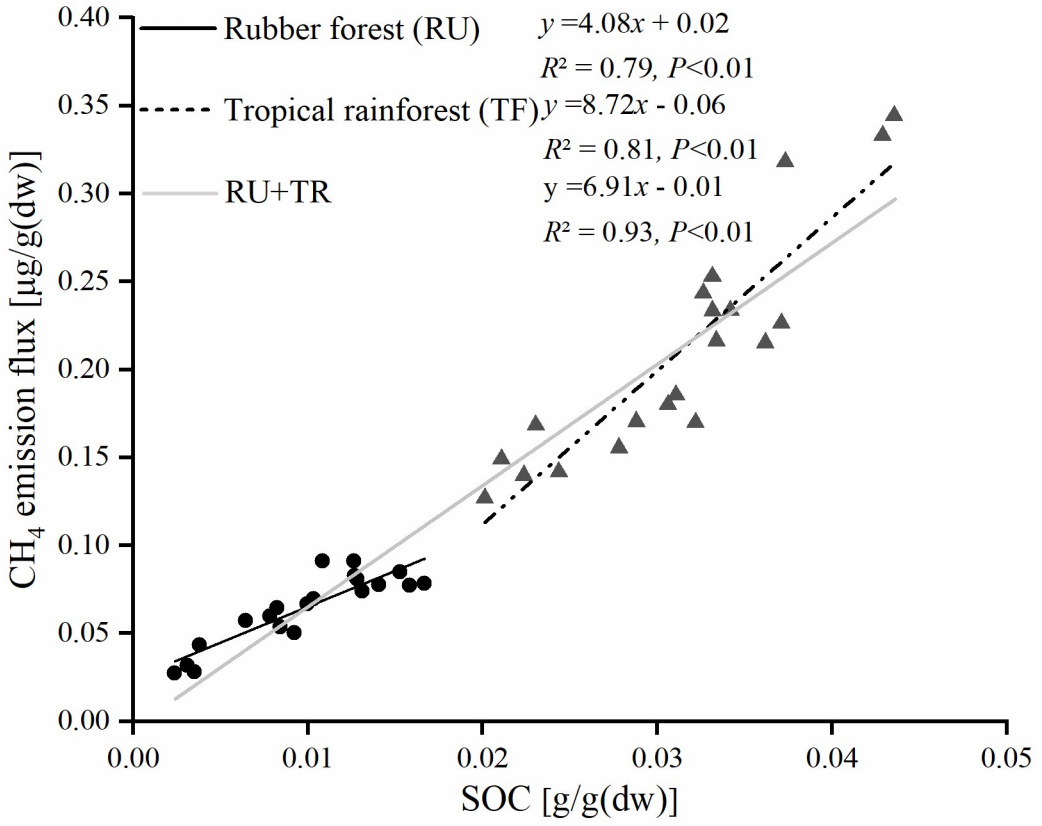

Fig 6. The relationships between soil NM-CH_4 emission fluxes and SOC in the tropical rainforest of Hainan.

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further clarified is which organic molecules or structures in the soil are involved in the process of soil NM-CH_4 release. In the study of plant materials, it is found that the methoxy groups in pectin and lignin can be used as precursors of NM-CH_4 production [18,20], which provides ideas for the study of soil NM-CH_4 sources. Mao et al. used solid-state nuclear magnetic resonance technology to study soil humic acid, and found it contains the methyl-containing functional groups such as alkoxy, alkyl and alkyl groups [36]. This indicated that the humic acid was one of the precursors of soil NM-CH_4 emissions. How large is soil NM-CH_4 emissions in tropical forest? Our results showed that the average NM-CH_4 emission fluxes were 6.91 μg per gram organic carbon in the tropical forest of Hainan. This study first reported the emission potential of soil NM-CH_4, which could provide basic data for understanding the production mechanisms and potential of soil NM-CH_4 in the tropical rainforest. In fact, the production of NM-CH_4 in the natural environment is the result of combined different factors (such as temperature, oxygen, soil water, etc.).

Conclusion

The NM-CH_4 could be produced in tropical rainforest soils under some condition. The NM-CH_4 emission would last about 229 h, and it exhibits a linear increase at first 24 h, but the increase rates decreased gradually as the incubation time increasing. The emission rates of NM-CH_4 under aerobic condition was higher than that under anaerobic condition at high temperature environment. The high temperature and H_2O_2 concentrations could significantly promote the emission of NM-CH_4 in tropical rainforest soil. The increasing of soil water

contents from 0 to 100% could promote the NM-CH_4 emission, while the excessive high soil water content inhibited the NM-CH_4 emission. There was significant positive correlation between SOC and NM-CH_4 emissions flux, which indicated that a great potential for NM-CH_4 emissions in tropical rainforest soils with high SOC.

In-depth research on NM-CH_4 plays an important role in further understanding the emission mechanism of soil CH_4 and more accurate prediction of climate change. However, the current researches on soil NM-CH_4 mainly concentrated on indoor incubation. How to conduct field in-situ observation (For example, two gaseous chemical reagents, monochloromethane and difluoromethane, are often used as inhibitors of CH_4 producing bacteria and CH_4 oxidizing bacteria, respectively. Can the two gases be directly used in the field and to achieve the purpose of inhibiting biomethane, thereby determining the release rate of NM-CH_4 under natural conditions), intuitively estimate its proportion to total CH_4, and explore more emission substrates and emission paths (For example, can the emission substrate and path be determined by the method of element labeling) still need to be further studied. This study found that temperature and moisture have a significant impact on NM-CH_4 emissions. The high-temperature and high-humidity soil environment of tropical forests, coupled with abundant organic carbon storage, can be considered as the best site for in-situ observation of microbial CH_4 and NM-CH_4. As we all know, model simulation is the best way to predict the current global change trend. If the process of NM-CH_4 is ignored, it may further increase the uncertainty of the regional and global carbon cycles predicted by current biogeochemical models. Therefore, based on the research that can distinguish between microbial CH_4 and NM-CH_4, algorithms for the production and emission of NM-CH_4 can be developed, verified, and integrated into the land surface model to better understand and predict large temporal and spatial changes.

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