



Rubber-leguminous shrub systems stimulate soil N₂O but reduce CO₂ and CH₄ emissions

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ARTICLE INFO

Keywords:

Greenhouse gases
Rubber
Carbon dioxide
Nitrous oxide
Methane
Xishuangbanna

ABSTRACT

Rubber plantations represent 13 million hectares in the world. Many researchers have focused on the environmental sustainability of rubber plantations, with rubber-legume systems better approach for ameliorating soil environments. This study investigated the effect of introducing *Flemingia macrophylla* (a leguminous shrub) to different-aged rubber plantations on the emissions of CO₂, N₂O, and CH₄. As trees aged in the rubber plantations, the annual emissions of CO₂, N₂O, and CH₄ significantly decreased. The introduction of *F. macrophylla* to different-aged rubber plantations significantly decreased CO₂ and CH₄ flux but increased N₂O flux. The CO₂ and N₂O fluxes were mainly affected by soil temperature at 10 cm depth, and CH₄ flux was mainly affected by both soil water content in the 0–10 cm soil layer and soil temperature at 10 cm depth. Compared to the same-aged rubber plantations, annual total CO₂ flux of young and mature rubber-*F. macrophylla* plantations decreased 154,000 and 64,000 kg ha⁻¹, CH₄ flux decreased 0.50 and 0.78 kg ha⁻¹ (17.0 and 26.5 kg CO₂ eq ha⁻¹), and N₂O flux increased 0.15 and 0.55 kg ha⁻¹ (44.7 and 163.9 kg CO₂ eq ha⁻¹), respectively. The rubber-leguminous shrub systems significantly improved soil organic carbon sequestration rate, relative to the same aged rubber plantations. In conclusion, the emissions of CO₂, N₂O and CH₄ decreased as the trees aged in the rubber plantations, and rubber-leguminous shrub systems could mitigate local climate warming by reducing greenhouse gas emissions and improving soil organic sequestration rate.

1. Introduction

In recent decades, rubber (*Hevea brasiliensis*) plantations have been replacing primary forest at a dramatic speed in Southeast Asia, as a result of high prices for rubber, and rubber plantations represent 13 million hectares in the world (de Blécourt et al., 2014; Fox et al., 2014; Song et al., 2014). Compared with primary forests, large areas of rubber monoculture have resulted in negative ecological effects, including reduced biodiversity (Li et al., 2007), lower total biomass (Li et al., 2008), and decreased soil quality (Fox et al., 2014; Liu et al., 2019a). The conversion of natural forests to rubber plantations decreases annual surface soil CO₂ fluxes, and weakens CH₄ sink function (Goldberg et al.,

2017; Lang et al., 2020). Tropical rainforests are converted to chemical fertilized rubber plantations, the increase of N₂O emissions may enhance local climate warming, and soil temperature, soil moisture and soil NH₄⁺-N control the variations in N₂O flux (Zhou et al., 2016). In the humid tropics, agroforestry uptakes less atmospheric CH₄ than forests (Mutuo et al., 2005).

In recent years, numerous rubber-based agroforestry systems, using a biological approach to enhance ecosystem services, have been developed, including improving system productivity and modifying environmental conditions (Jose, 2009, 2012; Liu et al., 2018). Intercropping legume plants with rubber trees can benefit rubber trees own higher N supply, reduce N inputs, and mitigate oil acidification processes,

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<https://doi.org/10.1016/j.foreco.2020.118665>

Received 29 September 2020; Accepted 30 September 2020

Available online 12 October 2020

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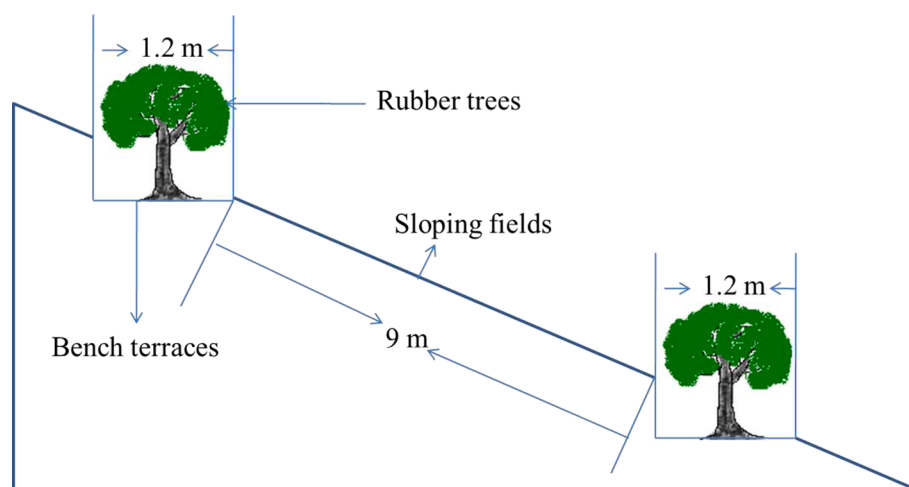


Fig. 1. Schematic presentation of rubber tree planting pattern.

therefore, legumes generally are considered as better intercropping plants in rubber plantations (Liu et al., 2018, 2019b; Wu et al., 2016). Legumes produce abundant amounts of easily decomposable litter and increase supply of N for soil via biological N fixation (Fox et al., 2014). Long-term *Chamaecrista rotundifolia* and *Arachis pintoii* mulching significantly increases total phosphorus, available nitrogen, soluble organic carbon, and influences bacterial communities of persimmon orchards in subtropical and tropical China (Zhong et al., 2018). Legumes may improve arbuscular mycorrhizal fungi abundance and alleviate soil P deficiencies in a karst grassland ecosystem (Xiao et al., 2019). Legumes reduce the emissions of GHG, and they can release 5–7 times less GHG per unit area compared with other crops (Bai et al., 2018). Legumes planted in grassland can mitigate climate change by reducing crop demand for N-fertilisers (Barneze et al., 2020). The introduction of nitrogen-fixing legumes to cereal-based crop rotations reduces the input of nitrogen fertilizers and may mitigate soil emissions of N₂O (Schwenke et al., 2015). Intercropped maize–soybean systems create a cooler and drier environment that was less favorable for denitrification, and reduce N₂O emissions compared to maize monoculture in the semi humid area of China (Shen et al., 2018). *Flemingia macrophylla* is a perennial multipurpose shrub legume, known for its various therapeutic uses, a supplementary feed, weed control, and high N-fixing capacity. Rubber-*F. macrophylla* intercropped systems have been widely established in rubber plantations area of China (Chen et al., 2017; Liu et al., 2019b).

Field GHG emissions are affected by the age of oil palm mainly due to the change of soil properties (Kusin et al., 2017). Soil pH and available P decreased as the rubber plantations aged, and soil water content

increased as the rubber plantations aged (Liu et al., 2019a, 2019b). The biomass accumulation of rubber and rubber-*F. macrophylla* plantations is affected by their plantation age (Zhang et al., 2016). Therefore, GHG emissions in rubber and rubber-*F. macrophylla* systems would be affected by their plantation age. However, the effect of different-aged rubber and rubber-*F. macrophylla* systems on CO₂, N₂O, and CH₄ emissions from soils is unknown. Therefore, this study aimed to: (1) compare the differences in CO₂, N₂O and CH₄ emissions between young and mature rubber plantations; (2) determine the influence of introducing *F. macrophylla* to different-aged rubber plantations on CO₂, N₂O and CH₄ emissions; (3) elucidate the main factors affecting CO₂, N₂O and CH₄ emissions in rubber and rubber-*F. macrophylla* plantations.

2. Materials and methods

2.1. Study site

The experimental site was located in Xishuangbanna (21°33'N, 101°28'E) in southwest China. The region is characterized by a typical southwest tropical monsoon climate with clear variation between the wet and dry periods. The annual mean temperature is 21.5 °C and mean annual precipitation is about 1500 mm. The annual precipitation was 1314.9 mm and the annual mean air temperature was 22.7 °C in 2016. There are three seasons: (1) dry, hot season from April to May with high temperatures and little rain; (2) rainy season from June to October with hot weather and rain accounting for 85% of annual falls; and (3) foggy, cool season from November to February with little rain but heavy fog in the morning and evening, which could compensate for insufficient water

Table 1

Soil properties at 0–10 cm soil depth in January 2016 (mean ± SD, $n = 3$) and total biomass for different-aged rubber and rubber-*Flemingia macrophylla* plantations in 2014.

	Soil pH	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	Exchangeable Al (cmol kg ⁻¹)	NH ₄ ⁺ (mg kg ⁻¹)	NO ₃ ⁻ (mg kg ⁻¹)	AP (mg kg ⁻¹)	BD (g cm ⁻³)	Biomass (t ha ⁻¹)	Crown density (%)
10R	5.89 ± 0.09a	12.64 ± 0.64b	1.44 ± 0.05b	0.26 ± 0.05c	4.53 ± 0.75c	0.02 ± 0.01b	2.7 ± 0.37b	1.42 ± 0.07a	47.3	42.7 ± 2.5b
10RF	5.97 ± 0.02a	13.66 ± 0.56ab	1.50 ± 0.02b	0.24 ± 0.04c	4.54 ± 0.34c	0.19 ± 0.07b	5.05 ± 0.49a	1.29 ± 0.04b	88.8	43.5 ± 2.3b
22R	5.03 ± 0.07b	14.40 ± 0.36a	1.62 ± 0.05a	3.35 ± 0.04a	9.70 ± 1.16a	0.10 ± 0.04b	1.59 ± 0.59c	1.41 ± 0.03a	101.3	83.5 ± 4.0a
22RF	5.24 ± 0.20b	14.40 ± 0.28a	1.60 ± 0.03a	0.96 ± 0.16b	6.99 ± 0.51b	1.90 ± 0.33a	2.69 ± 0.28b	1.30 ± 0.03b	117.4	86.0 ± 3.6a

10R: rubber plantations established in 2006; 22R: rubber plantations established in 1994; 10RF: *Flemingia macrophylla* introduced to 10R in 2010; 22RF: *Flemingia macrophylla* introduced to 22R in 2010. The total biomass of the different-aged rubber and rubber-*Flemingia macrophylla* plantations was measured in 2014 (Wang, 2015). SOC: soil organic carbon; TN: soil total nitrogen; AP: soil available phosphorus; BD: soil bulk density. Values within a column followed by the same letter do not significantly differ at $P \leq 0.05$.

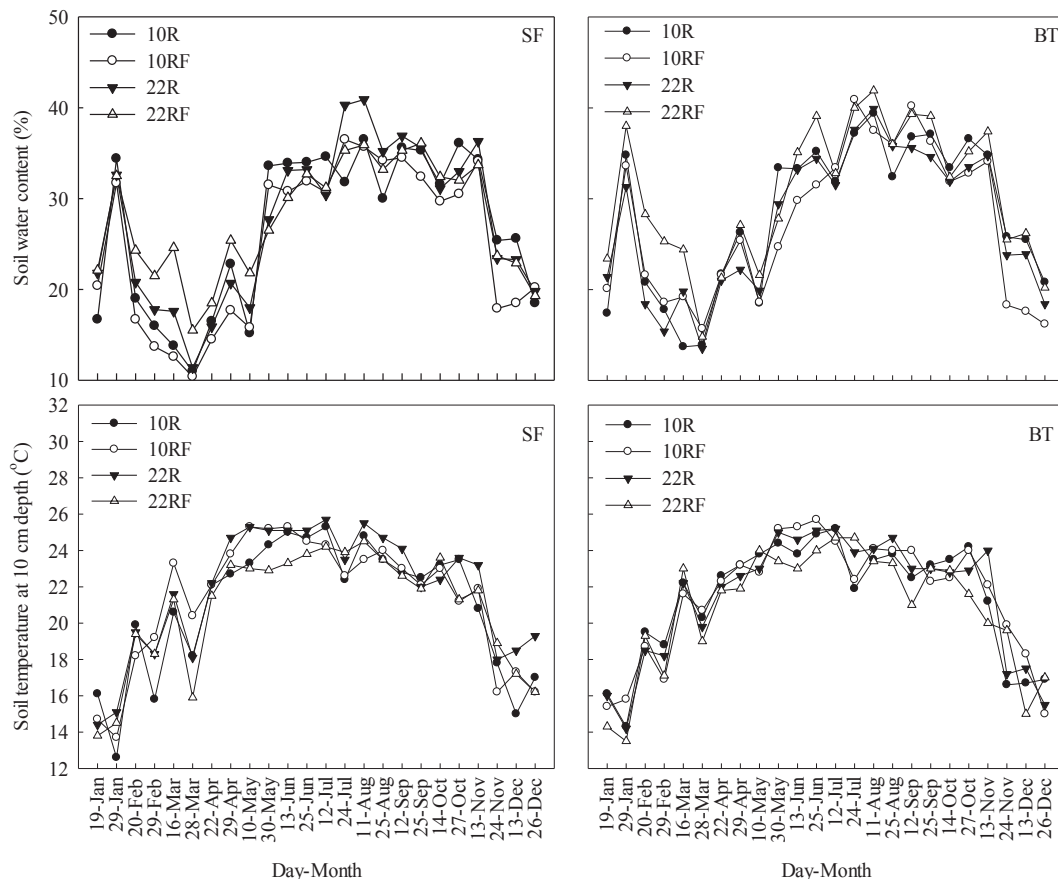


Fig. 2. Soil water content in the 0–10 cm soil layer and soil temperature at 10 cm depth between 9:00 and 11:00 A.M. when gas samples were collected in different-aged rubber and rubber–*Flemingia macrophylla* plantations. 10R: rubber plantations established in 2006; 22R: rubber plantations established in 1994; 10RF: *Flemingia macrophylla* introduced to 10R in 2010; 22RF: *Flemingia macrophylla* introduced to 22R in 2010. SF: sloping fields in the rubber and rubber–*Flemingia macrophylla* plantations; BT: bench terraces in the rubber and rubber–*Flemingia macrophylla* plantations.

derived from rain.

2.2. Experimental design

In 1991 and 2003, tropical forests with the same slope aspect, ranging from 20 to 25°, were deforested in Xishuangbanna and replaced with sugarcane (*Saccharum officinarum*); in May 1994 and 2006, respectively, two adjacent rubber tree plantations replaced the sugarcane at these sites. Rubber trees were planted at a density of 450 plants ha⁻¹ with rows 8.0 m apart and trees 2.5 m apart (Fig. 1). In 2010, *F. macrophylla* was introduced in the two different-aged rubber plantations at a density of 10,830 plants ha⁻¹ with rows 1.0 m apart and plants 0.8 m apart. In January 2016, four stand types—10-year-old rubber plantations (10R, rubber plantations established in 2006), 10-year-old rubber–*F. macrophylla* plantations (10RF, *F. macrophylla* introduced to 10R in 2010), 22-year-old rubber plantations (22R, rubber plantations established in 1994), and 22-year-old rubber–*F. macrophylla* plantations (22RF, *F. macrophylla* introduced to 22R in 2010)—were selected in these sites. Each stand type was at least 500 m apart. Three replicates plots, each with a sampling area of 10 × 10 m and at least 50 m apart, were established for each stand type. The soil is rhodic ferralsol (according to FAO taxonomy). The sand, silt, and clay contents are 15.4–18.3%, 50.3–50.9%, and 31.4–33.7% for the two different-aged rubber and rubber–*F. macrophylla* plantations. In accordance with local practices, rubber plantations <3-years-old received 0.06 kg N, 0.03 kg P₂O₅, and 0.03 kg K₂O in each tree trench (100 cm long × 20 cm wide × 20 cm deep) in May and October each year. Rubber plantations >3-years-old received 0.12 kg N, 0.06 kg P₂O₅, and 0.06 kg K₂O in each tree

trench (Liu et al., 2018). *F. macrophylla* in the mixed systems was cut in December each year and left as ground cover.

The species of understory vegetation in 10R was dominated by *Lobelia nummularia*, *Cyclosorus acuminatus*, *Pogonatherum panicum*, *Rungia pectinate*, *Crassocephalum rubens*, *Cyathocline purpurea*, *Melastoma malabathricum*, *Blumea fistulosa*, *Blumea axillaris*, *Selaginella uncinata*, *Ageratina Adenophora*, *Thunbergia grandiflora*, *Bidens Pilosa*, *Kyllinga brevifolia*, *Sinodolichos lagopus*, *Hedyotis*, *Dioscorea bulbifera* and *Selaginella helferi*; 10RF was dominated by *F. macrophylla*; 22R was dominated by *Hedyotis auricularia*, *Ottlochloa nodosa*, *Curculigo capitulate*, *Imperata cylindrica*, *Fargesia caduca*, *Thysanolaena latifolia* and *Apluda mutica*; 22RF was dominated by *F. macrophylla*, *Hedyotis auricularia*, *Ottlochloa nodosa*, *Curculigo capitulate*.

2.3. Measurement of CO₂, N₂O, and CH₄ fluxes

The CO₂, N₂O, and CH₄ fluxes were measured using static chambers and gas chromatography techniques (Wang and Wang, 2003). Each static chamber is comprised of a base box (0.50 m × 0.50 m × 0.10 m) and chamber box (0.50 m × 0.50 m × 0.50 m). A U-shaped groove (50 mm wide and 50 mm deep) on the base box holds the removable chamber. Both the base and chamber boxes were made from dark polyvinyl chloride (PVC) boards (5 mm thick). A small fan was installed inside the chamber box to homogenize the gas.

Per plot for each stand type had two boxes, one on the slope and one on the terrace, taking the micro-topography in the rubber plantations into consideration for more accurate measurements of CO₂, N₂O and CH₄ flux in soils, and litterfall in base box was not removed (Dong et al.,

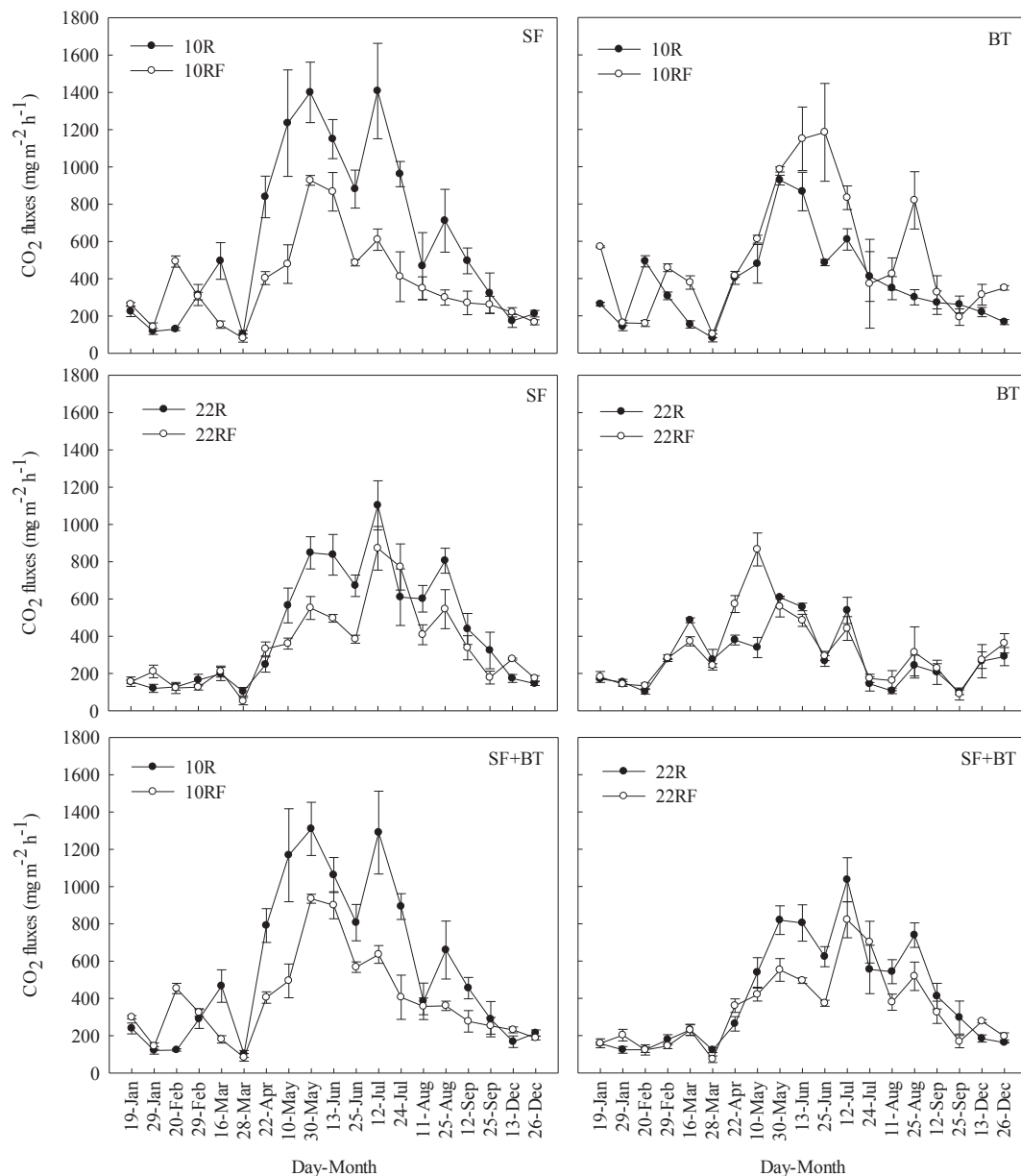


Fig. 3. CO₂ fluxes in different-aged rubber and rubber-*Flemingia macrophylla* plantations. 10R: rubber plantations established in 2006; 22R: rubber plantations established in 1994; 10RF: *Flemingia macrophylla* introduced to 10R in 2010; 22RF: *Flemingia macrophylla* introduced to 22R in 2010. SF: sloping fields in the rubber and rubber-*Flemingia macrophylla* plantations; BT: bench terraces in the rubber and rubber-*Flemingia macrophylla* plantations. Bars are standard deviations of the mean ($n = 3$).

2015; Zhou et al., 2016). The calculation for one replication is as follows:

$$F = \mu_1 \times F_{\text{Bench terraces}} + \mu_2 \times F_{\text{Sloping fields}}$$

where $F_{\text{Bench terraces}}$ and $F_{\text{Sloping fields}}$ is the total CO₂ flux ($\text{mg m}^{-2} \text{h}^{-1}$), N₂O flux ($\mu\text{g m}^{-2} \text{h}^{-1}$), or CH₄ flux ($\mu\text{g m}^{-2} \text{h}^{-1}$) of terraces and slopes for one replicate; μ_1 and μ_2 are two constants (0.1176 and 0.8824, respectively) calculated from the proportion of occupied area of terraces and slopes to replication area. The base box was inserted 0.05 m into the soil. Before gas sampling, the U-shaped groove was filled with distilled water to a depth of approximately 20 mm, and the chamber box was placed on the base box (Zhang et al., 2014). Gas samples were taken with a 60 mL plastic syringe attached to a 3-way stopcock at 0, 15, 30, 45 and 60 min after closing the chamber. The gas samples were placed in the shade to protect the syringes from direct sunlight and to minimize temperature changes during sampling (Liu et al., 2011). The chamber was removed

after the sampling was completed. The concentrations of CO₂, N₂O, and CH₄ in the samples were analyzed in the laboratory using gas chromatography (Agilent 4890 D, Agilent Technologies, CA, USA) within 24 h to avoid any changes in gas concentration in the plastic syringes (Wang and Wang, 2003). The gas passes through the equipped nickel catalytic system to convert CO₂ into CH₄ at 375 °C, and then uses the FID to detect the CH₄ signal; the CH₄ in the gas sample is directly detected by the FID; and the N₂O in the gas sample is analyzed by ECD (Zou et al., 2002). All gas samples were collected between 9:00 and 11:00 A.M. to minimize the change in soil CO₂, N₂O and CH₄ fluxes resulting from diurnal variation in environmental factors; it is generally accepted that CO₂, N₂O, and CH₄ fluxes during this period are close to the daily mean (Tang et al., 2006). GHG flux was calculated using the rate of change in GHG concentration inside the chamber, which was estimated as the slope of the linear regression between concentration and time (Chen et al., 2010); coefficients of determination (R^2) of the linear regression >0.80

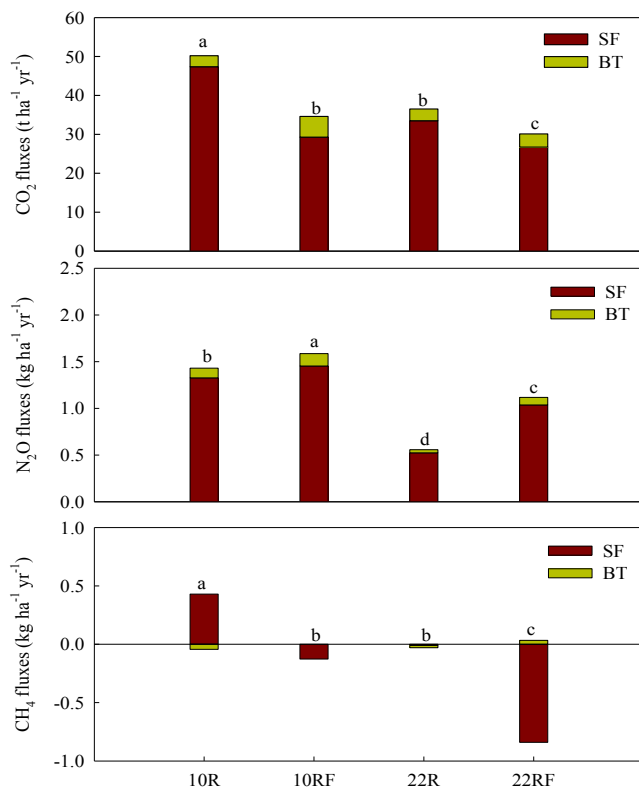


Fig. 4. Total CO₂, N₂O, and CH₄ fluxes in different-aged rubber and rubber-*Flemingia macrophylla* plantations. 10R: rubber plantations established in 2006; 22R: rubber plantations established in 1994; 10RF: *Flemingia macrophylla* introduced to 10R in 2010; 22RF: *Flemingia macrophylla* introduced to 22R in 2010. SF: sloping fields in the rubber and rubber-*Flemingia macrophylla* plantations; BT: bench terraces in the rubber and rubber-*Flemingia macrophylla* plantations. Different letters indicate significant differences at $P \leq 0.05$.

were considered valid data in our study. The CO₂, N₂O, and CH₄ fluxes were measured twice per month from January to December 2016.

In our study, biomass of the two different-aged rubber and rubber-*F. macrophylla* plantations in 2014 are presented in Table 1, which was measured by Wang (2015). Yearly cumulative fluxes of CO₂, N₂O and CH₄ by cumulating the average daily cumulative fluxes of CO₂, N₂O and CH₄ * 365. For the calculation of average daily cumulative fluxes, we transfer the unit of fluxes from mg/μg m⁻² h⁻¹ to mg/μg m⁻² day⁻¹ according to the average of all sampled data per year.

2.4. Soil sampling and determination of soil parameters

In each replicate plot for each stand type, nine core soil samples were taken in the 0–10 cm soil layer in January 2016 and 2017, which were combined into a composite sample. Once combined, the soil was passed through a 2-mm sieve to remove roots, gravel, and stones for analysis of soil chemical properties. The air-dried samples were >2.0 mm for determination of soil pH, available phosphorus (AP), exchangeable Al, NH₄⁺, and NO₃⁻. The sieved samples were sieved again (at <0.25 mm) for the determination of soil organic carbon (SOC) and total nitrogen (TN). Soil pH was measured in a 1: 2.5 soil: water suspension with a pH meter (PHS-3E, Leici, China). Soil available P was extracted using 0.025 mol L⁻¹ HCl and 0.03 mol L⁻¹ NH₄F, then determined colorimetrically (Anderson and Ingram, 1989). Soil exchangeable Al was extracted with 1 mol L⁻¹ KCl and determined by titration with NaOH (Hou et al., 2012). NH₄⁺ and NO₃⁻ contents were extracted with 2 mol L⁻¹ KCl and analyzed with an Auto Analyzer 3 (SEAL Analytical GmbH, Germany) (Liu et al., 2013). SOC and TN were analyzed by dry combustion with an elemental analyzer (Elementar Analysensysteme GmbH, Germany). Soil

moisture in the 0–10 cm soil layers (TD10R00, Campbell Scientific, USA) and temperatures at 10 cm depths were monitored in each chamber in situ while gas samples were collected. The soil temperature at 10 cm depth in stand types was recorded at 08:00, 14:00 and 20:00 h daily for three consecutive days in the middle of January, April, August, and November in 2016. The mean daily soil temperature was calculated as the average of the three daily reading. The soil bulk density (BD) at 0–10 cm depth was measured according to Robertson et al. (1999). 3 soil core samples in each replicate plot were collected in January 2016, immediately weighed, dried at 105 °C to a constant weight, and then reweighed.

The soil organic carbon sequestration rate (SOCSR) was calculated as follows (Liu et al., 2015):

$$\text{SOCSR (kg C ha}^{-1}\text{ yr}^{-1}) = (\text{SOC}_t - \text{SOC}_0) \times \gamma \times (1 - \delta_{2\text{mm}}/100) \times 10 \times 100$$

C_t and SOC₀ are the soil organic carbon contents measured in January 2017 and 2016, respectively; γ and δ_{2mm} are the average bulk density and the gravel content (>2 mm) of the soil (0–10 cm), respectively. The number 10 represents the thickness of the topsoil in four stand types.

2.5. Statistical analysis

Statistical analysis was conducted using the SAS software package (SAS Institute, 1990). One-way ANOVA was used to examine differences in SOC, TN, exchangeable Al, NH₄⁺, NO₃⁻, AP, BD, SOCSR, crown density of rubber trees, daily soil temperature in different-aged rubber and rubber-*Flemingia macrophylla* plantations ($P \leq 0.05$), and two-way ANOVA for CO₂, N₂O and CH₄ fluxes within the rubber and rubber-*F. macrophylla* plantations with age and N-fixing species as factors ($P \leq 0.05$). Exponential growth analysis was performed with the CO₂, N₂O and CH₄ fluxes rate as the soil temperature at 10 cm depth. The relationships between soil water content in the 0–10 cm soil layers and CO₂, N₂O, and CH₄ flux were tested using Pearson's correlation analysis.

3. Results

3.1. Soil properties, water content, temperature, and CO₂, N₂O and CH₄ fluxes

SOC, TN, exchangeable Al, and NH₄⁺ increased, and soil pH and available P decreased as the trees aged in the rubber and rubber-*F. macrophylla* plantations (Table 1). The introduction of *F. macrophylla* to mature rubber plantations significantly increased soil NO₃⁻ and available P and decreased exchangeable Al and soil bulk density. The introduction of *F. macrophylla* to young rubber plantations decreased soil water contents in the 0–10 cm soil layers (Fig. 2). While the introduction of *F. macrophylla* to mature rubber plantations increased soil water contents. There were no obvious differences in soil temperatures at 10 cm depth between 9:00 and 11:00 A.M in four stand types (Fig. 2).

The rainy season produced consistently higher CO₂ flux in the bench terraces and sloping fields in all plantation treatments than the dry season (Fig. 3). The introduction of *F. macrophylla* to the different-aged rubber plantations decreased CO₂ flux in the sloping fields in the rainy season. Annual total CO₂ flux significantly decreased as the trees aged in the rubber and rubber-*F. macrophylla* plantations (Fig. 4). There were no obvious differences between plantation treatments in total CO₂ flux in the bench terraces. The introduction of *F. macrophylla* to the different-aged rubber plantations decreased total CO₂ flux in the sloping fields.

May to August produced consistently higher N₂O flux in the bench terraces and sloping fields in 10R and 10RF than other months (Fig. 5). N₂O flux in 22R and 22RF had no obvious changes in the bench terraces and sloping fields over the entire experiment. The introduction of

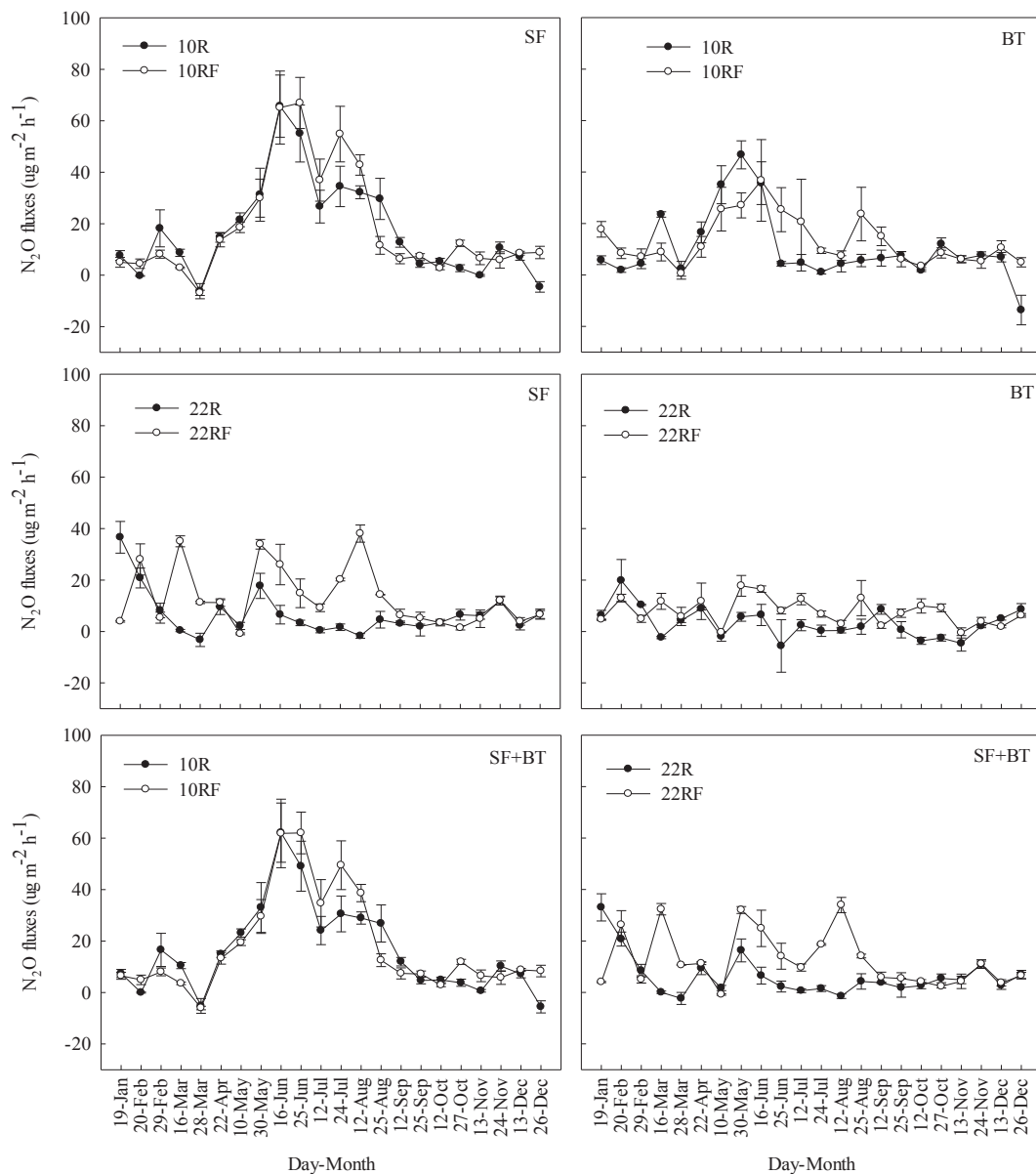


Fig. 5. N_2O fluxes in different-aged rubber and rubber-*Flemingia macrophylla* plantations. 10R: rubber plantations established in 2006; 22R: rubber plantations established in 1994; 10RF: *Flemingia macrophylla* introduced to 10R in 2010; 22RF: *Flemingia macrophylla* introduced to 22R in 2010. SF: sloping fields in the rubber and rubber-*Flemingia macrophylla* plantations; BT: bench terraces in the rubber and rubber-*Flemingia macrophylla* plantations. Bars are standard deviations of the mean ($n = 3$).

F. macrophylla to the different-aged rubber plantations increased N_2O flux in the sloping fields from May to August. Annual total N_2O flux significantly decreased as the trees aged in the rubber and rubber-*F. macrophylla* plantations (Fig. 4). There were no obvious differences in total N_2O flux in the bench terraces between plantation treatments. The introduction of *F. macrophylla* to the different-aged rubber plantations increased the total N_2O flux in the sloping fields.

The soils in the different-aged rubber and rubber-*F. macrophylla* plantations were a net CH_4 sink in the dry season and a net CH_4 source in the rainy season (Fig. 6). The introduction of *F. macrophylla* decreased CH_4 flux in the sloping fields of young rubber plantations from June to September and mature rubber plantations from May to July. Annual total CH_4 flux decreased as the trees aged in the rubber and rubber-*F. macrophylla* plantations (Fig. 4). There were no obvious differences in total CH_4 flux in the bench terraces between plantation treatments. The introduction of *F. macrophylla* to the different-aged rubber plantations increased atmospheric CH_4 uptake.

SOCSR significantly decreased as the trees aged in the rubber and rubber-*F. macrophylla* plantations (Fig. 7). The introduction of *F. macrophylla* to the young and mature rubber plantations significantly improved SOCSR.

3.2. Correlation of CO_2 , N_2O , and CH_4 fluxes with temperature and moisture

Soil water content had a significant positive correlation with CH_4 flux ($P < 0.0001$) (Fig. 8). A significant exponential growth relationship was found between soil temperature at 10 cm depths and CO_2 , N_2O , and CH_4 flux in the bench terraces and sloping fields ($P < 0.0001$) (Fig. 8).

The stepwise regressions for evaluating the relationships between soil water content, soil temperature at 10 cm depth, and CO_2 , N_2O , and CH_4 flux are below. The relationship of CO_2 , N_2O , and CH_4 flux to soil water content and soil temperatures at 10 cm depth was:

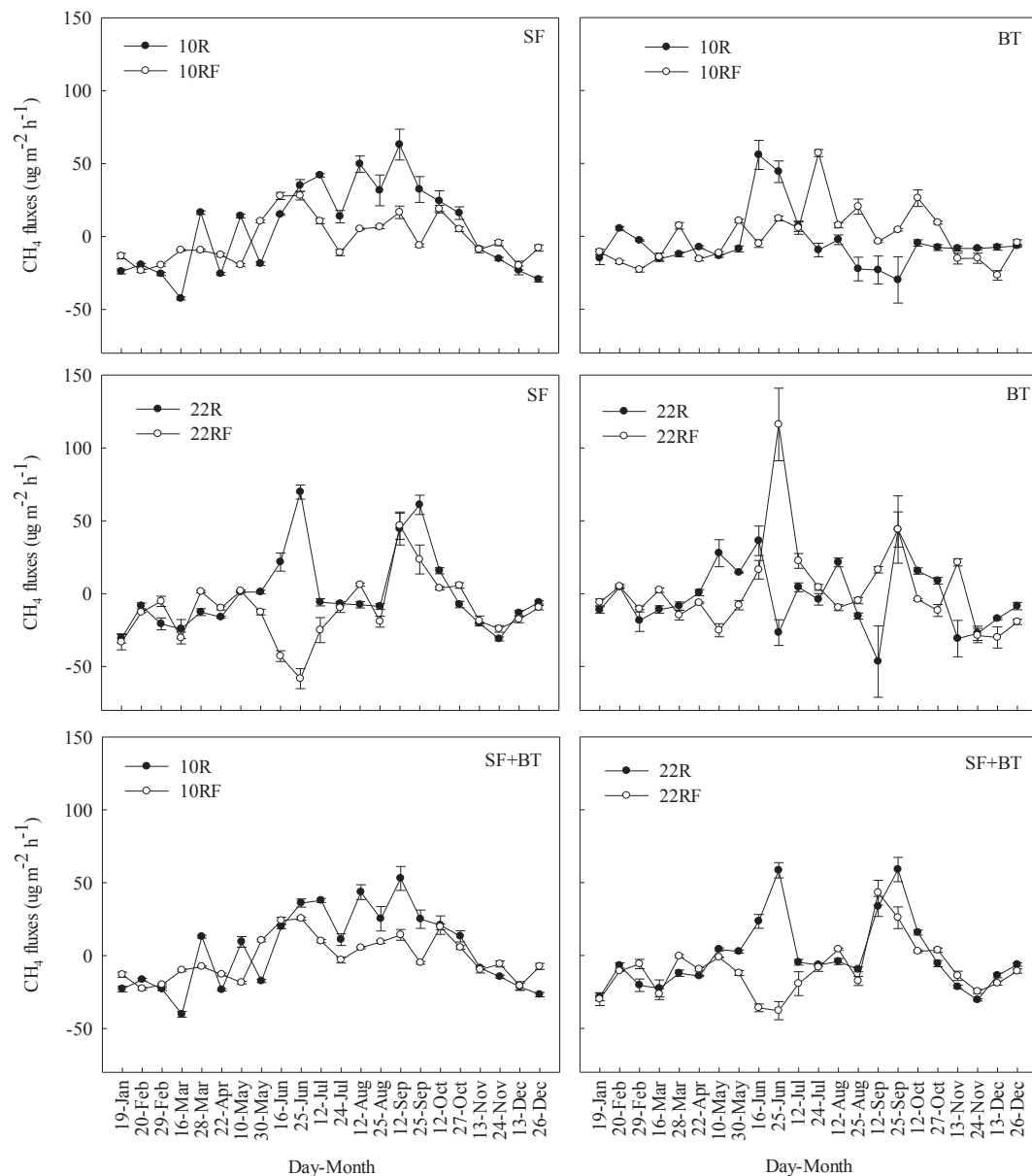


Fig. 6. CH₄ fluxes in different-aged rubber and rubber-*Flemingia macrophylla* plantations. 10R: rubber plantations established in 2006; 22R: rubber plantations established in 1994; 10RF: *Flemingia macrophylla* introduced to 10R in 2010; 22RF: *Flemingia macrophylla* introduced to 22R in 2010. SF: sloping fields in the rubber and rubber-*Flemingia macrophylla* plantations; BT: bench terraces in the rubber and rubber-*Flemingia macrophylla* plantations. Bars are standard deviations of the mean ($n = 3$).

$$YCO_2 = 0.558 \cdot T_{10} \quad (R^2 = 0.31, P < 0.001)$$

$$YN_2O = 0.263 \cdot T_{10} \quad (R^2 = 0.06, P < 0.001)$$

$$YCH_4 = 0.288 \cdot SWC + 0.218 \cdot T_{10} \quad (R^2 = 0.20, P < 0.001)$$

where YCO_2 , YN_2O , and YCH_4 are CO_2 , N_2O , and CH_4 flux, respectively, SWC is soil water content in the 0–10 cm soil layer, and T_{10} are soil temperatures at 10 cm depths, respectively. Therefore, CO_2 and N_2O fluxes were largely affected by soil temperature at 10 cm depth, and CH_4 flux was largely affected by soil water content in the 0–10 cm soil layer and soil temperature at 10 cm depth. CO_2 , N_2O and CH_4 fluxes were significantly affected by the ages of the plantations and the introduction of *F. macrophylla* (Table 2).

4. Discussion

The fluxes of CO_2 , CH_4 , and N_2O of woodland and shrubland soils are strongly influenced by soil water content and temperature (Zona et al.,

2013; Dou et al., 2016). In this study, no significant correlation was observed between CO_2 flux and soil water content in the sloping fields in rubber plantations. CO_2 flux was strongly influenced by soil temperature at 10 cm depth, which agrees with the findings of Wang et al. (2013), who suggested that soil respiration is closely related to soil temperature, but may not be directly related to soil moisture in rubber plantations in Hainan of China. In these regions, the mean annual precipitation is generally over 1500 mm, and soil water content is not a limiting factor for CO_2 emissions. The young rubber plantations had less crown density than mature rubber plantations (42.7% for 10R and 83.5% for 22R), and the sufficient light in young rubber plantations increased the biodiversity under the rubber trees and improved CO_2 emissions from soil compared to the mature rubber plantations. Plants generally show Al toxicity symptoms when soil exchangeable Al is $>2.0 \text{ cmol kg}^{-1}$ (Van Breemen et al., 1984). In the mature rubber plantations, soil exchangeable Al in the 0–10 cm soil layer was $3.35 \text{ cmol kg}^{-1}$, a high enough level to inhibit root development and reduce CO_2 emissions.

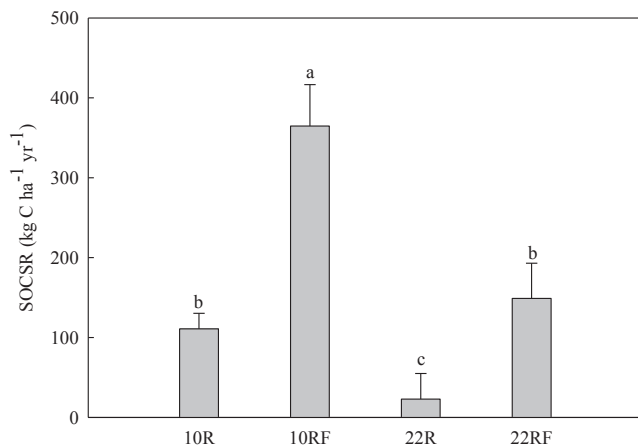


Fig. 7. Soil organic carbon sequestration rate (SOC SR) in different-aged rubber and rubber-*Flemingia macrophylla* plantations, from January 2016 to January 2017. 10R: rubber plantations established in 2006; 22R: rubber plantations established in 1994; 10RF: *Flemingia macrophylla* introduced to 10R in 2010; 22RF: *Flemingia macrophylla* introduced to 22R in 2010. Different letters indicate significant differences at $P \leq 0.05$. Bars are standard deviations of the mean ($n = 3$).

Root respiration accounts for 40–70% of the total soil respiration in forest ecosystems (Hanson et al., 2000). Fine roots of forest ecosystems contribute a large portion to the total root respiration due to their large biomass and rapid turnover (Fu et al., 2019). Huang (2018) reported that fine roots biomass of rubber trees was 2.32 and 1.78 t ha⁻¹ in the 0–60 cm soil layer for 15 and 20-year-old rubber plantations in Xishuangbanna of China, respectively. The high fine roots biomass of rubber trees in young rubber plantations may enhance CO₂ emissions from soils.

The introduction of *F. macrophylla* to the different-aged rubber plantations significantly decreased total CO₂ flux. The strong sprouting ability of *F. macrophylla* inhibited other plants growth under rubber trees (Fig. 9). Almost no other vegetation can survive in the young rubber-*F. macrophylla* plantations. The lower CO₂ emissions in different aged rubber-*F. macrophylla* plantations maybe due to the decrease of plant species under rubber trees. The decrease of plant species under rubber trees in rubber-*F. macrophylla* plantations would lead to the decrease of root respiration. In this study, CO₂ emissions increased exponentially with the increase of the soil temperature at 10 cm depths. We found that the introduction of *F. macrophylla* to the different-aged rubber plantations significantly decreased daily soil surface temperature at 10 cm depth (Table 3). It indicated that rubber-*F. macrophylla* plantations could significantly decrease CO₂ emissions from soil by reducing soil temperature.

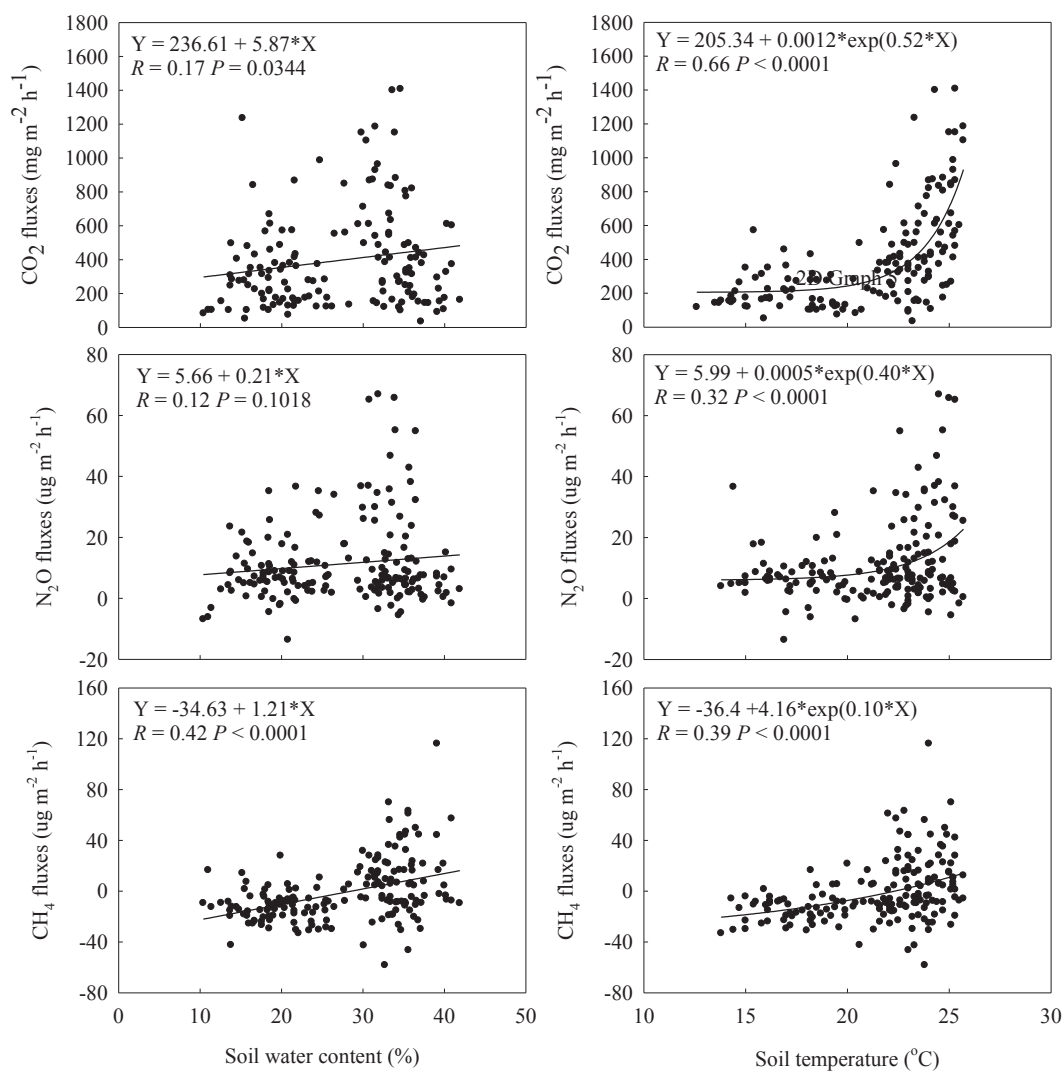


Fig. 8. Relationships between soil water content in the 0–10 cm soil layer, temperature at 10 cm depth, and CO₂, N₂O, and CH₄ fluxes. SF: sloping fields in the rubber and rubber-*Flemingia macrophylla* plantations; BT: bench terraces in the rubber and rubber-*Flemingia macrophylla* plantations.

Table 2

Two-way Analysis of Variance for CO₂, N₂O and CH₄ fluxes within the rubber and rubber-*Flemingia macrophylla* plantations with age and N-fixing species as factors.

		F-value	P-value
CO ₂	Age	6.81	0.010
	N-fixing species	10.06	0.002
	Age*N-fixing species	1.69	0.195
N ₂ O	Age	18.00	0.001
	N-fixing species	4.95	0.027
	Age*N-fixing species	1.643	0.201
CH ₄	Age	5.49	0.020
	N-fixing species	7.49	0.007
	Age*N-fixing species	0.343	0.558

We found that N₂O flux was more responsive to variations in soil temperature than soil water content indicating that N cycling processes are relatively sensitive to variation in soil temperature because of the abundant rainfall in the tropical regions. Our results agree with those of Yan et al. (2008), who reported that N₂O flux had positive correlation with soil temperature, and insensitive to soil moisture in a tropical seasonal rain forest. Total N₂O flux decreased significantly as the trees aged in the rubber plantations, as did soil available P and pH (Table 1), which altered bacterial communities and reduced the species richness of bacteria (Liu et al., 2019c). The decline in species richness of bacteria in the mature rubber plantations would mitigate nitrification and denitrification processes and reduce N₂O emissions. Long-term nitrogen-fixing legumes mulching significantly increases available nitrogen by improving rate of nitrification in subtropical and tropical China (Zhong et al., 2018). Legume residues can rapidly release large amounts of mineral N, and then produce N₂O by nitrification and denitrification (Sant'Anna et al., 2018). In a previous study, we found that the introduction of *F. macrophylla* to the different-aged rubber plantations increases NO₃⁻ by improving rate of nitrification, and simultaneously increases dissolved organic carbon content (DOC) (Liu et al., 2018). The increase of nitrification in rubber-*F. macrophylla* plantations would improve N₂O emissions from soil. Qin et al. (2019) reported that NO₃⁻ and DOC concentrations significantly affected N₂O flux in two acid forest soils, and alteration of soil NO₃⁻ and DOC concentrations affects the microbial (gene) community composition of nitrifiers and denitrifiers. The increase of soil NO₃⁻ and DOC concentrations in this study would promote the release of N₂O.

In this study, CH₄ flux was strongly influenced by soil water content and temperature at 10 cm depth, indicating that soil water content and temperature is an important environmental factor for CH₄ emissions in rubber plantations. The high SOC and microbial biomass carbon (MBC) in afforested lands are propitious to enhance CH₄ uptake capacity (Wu

et al., 2018). No-tillage with legume cover crops is an effective measure for SOC storage in a previously degraded subtropical soil (Veloso et al., 2018), and cereal-legume intercropping systems can increase microbial biomass in the rhizosphere (Tang et al., 2014). In previous report, SOC and MBC increased as trees aged in the rubber plantations (Liu et al., 2018). The high SOC and MBC in mature rubber plantations would increase CH₄ uptake. The activities of methane-oxidizing bacteria increase under aerobic conditions, and those of methane-producing bacteria increase under anaerobic environments (Iqbal et al., 2013; Nan et al., 2020). Chen et al. (2017) reported that the introduction of *F. macrophylla* to rubber plantations improved total soil porosity, soil mean weight diameter, and soil hydraulic conductivity, relative to rubber monoculture. In this study, we found that the introduction of *F. macrophylla* to the different-aged rubber plantations significantly decreased soil bulk density. The increase in soil aeration in rubber-*F. macrophylla* plantations would improve the activities of methane-oxidizing bacteria, and soils would absorb more CH₄ from the atmosphere.

Compared to the same-aged rubber plantations, annual total CO₂ flux of young and mature rubber-*F. macrophylla* plantations decreased 154,000 and 64000 kg ha⁻¹, CH₄ flux decreased 0.50 and 0.78 kg ha⁻¹ (17.0 and 26.5 kg CO₂ eq ha⁻¹, (IPCC, 2013)), and N₂O flux increased 0.15 and 0.55 kg ha⁻¹ (44.7 and 163.9 kg CO₂ eq ha⁻¹, (IPCC, 2013)), respectively. Therefore, decrease of CO₂ emissions is crucial for the reduction of greenhouse gas emissions in rubber plantations worldwide. Compared to the same-aged rubber plantations, the biomass of young and mature rubber-*F. macrophylla* plantations increased 87.7 and 15.9%, respectively, and which caused the increase of SOCSR.

5. Conclusion

The emissions of CO₂, N₂O and CH₄ decreased as the trees aged in the rubber plantations. The rubber-leguminous shrub systems significantly

Table 3

. Mean daily soil temperature at 10 cm depth in different-aged rubber and rubber-*Flemingia macrophylla* plantations in 2016 (mean ± SD, n = 3).

	January	April	August	November
10R	16.8 ± 1.0a	22.8 ± 0.9a	24.6 ± 0.4a	18.9 ± 0.1a
10RF	14.8 ± 0.3b	22.5 ± 0.5a	23.7 ± 0.2b	17.6 ± 0.2b
22R	16.8 ± 0.7a	23.0 ± 1.1a	24.5 ± 0.2a	19.0 ± 0.6a
22RF	14.6 ± 0.5b	22.1 ± 0.1a	23.6 ± 0.7b	17.4 ± 0.6b

10R: rubber plantations established in 2006; 22R: rubber plantations established in 1994; 10RF: *Flemingia macrophylla* introduced to 10R in 2010; 22RF: *Flemingia macrophylla* introduced to 22R in 2010. Values within a column followed by the same letter do not significantly differ at $P \leq 0.05$.



Fig. 9. *Flemingia macrophylla* inhibits other plants growth under rubber trees. A: Sloping fields in the rubber plantations; B: Sloping fields in the rubber-*Flemingia macrophylla* plantations.

improved soil organic carbon sequestration rate and reduced greenhouse gas emissions, relative to the same-aged rubber plantations. Rubber-leguminous shrub systems should be popularized in the rubber planting area for reducing greenhouse gas emissions. The age of mature rubber plantations could be extended by improving management practices to decrease greenhouse gas emissions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to acknowledge Dr. Yuntong Liu for his technical assistance, and the Public Technology Service Center of Xishuangbanna, Chinese Academy of Sciences and National Forest Ecosystem Research Station at Xishuangbanna for their support with soil analyses and meteorological data, respectively. This study was funded by the National Natural Science Foundation of China (31470639), the Natural Science Foundation of Yunnan Province (2017FB059 and 2016FA047), and the Key Program of Chinese Academy of Sciences (KFZD-SW-312).

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