Revision EGUSPHERE-2025-5065

Point by point response to the reviewers:

First of all, we would like to thank the editor and the reviewers for the positive and constructive comments. We carefully revised and improved the article. The followings are response to the comments of reviewers. The line number below is indicated based on the **clean version.**

General Comments

The manuscript presents valuable insights from a two-year measurement campaign of CO_2 fluxes in a tea plantation in China. The study compares plots subjected to different intercropping treatments, providing an interesting perspective by distinguishing emissions from tea rows and inter-row spaces. The discussion is comprehensive; however, additional experimental details would enhance the reader's ability to interpret the results. Aside from this, I have only a few minor comments (see below). Overall, the paper falls within the scope of *Biogeosciences* and merits publication after these minor revisions are addressed.

Response: Thank you very much for your positive comments. We have revised the manuscript and answered the questions point by point. The line number below is indicated based on the clean version.

Specific Comments:

Abstract

1. Lines 28–31: These absolute values are difficult to interpret without broader context regarding tea plantation emissions. Readers will likely be more interested in relative changes compared to control plots, as mentioned in line 41. Please provide relative changes for the total flux as well (see comment on line 41).

   Response: Thank you for the comments Your viewpoint is indeed more accurate. We have removed the average values and replaced them with the relative changes in average fluxes. We revised the description (Lines 29–32).

   "Average tea-row fluxes were 8.7% and 9.5% lower under SM and HM, respectively, compared to CK, indicating emission reductions with intercropping. In contrast, average inter-row fluxes increased by 19.4% under SM and 7.7% under HM, demonstrating pronounced spatial contrasts."

2. Lines 32–33: Specify for which plots midday peaks occurred.

   Response: Thank you for the comments. While diurnal variations differed slightly between tea rows and inter-rows and among different treatments, it is worth noting that, overall, the peak fluxes predominantly occurred around midday. We revised the description (Lines 32–35).

   "Diurnal patterns generally exhibited midday peaks (12:00–14:00), especially in summer and autumn across all tea-rows, and short-term CO_2 pulses were triggered by field operations such as fertilization and pruning."

3. Line 41: The relative changes reported do not correspond to the values in lines 28–31. Be more precise which aggregates are shown and used for the relative changes.

   Response: Thank you for the comments. The reported percentage changes are based on the total annual emissions (derived from the fluxes), rather than on the average flux values alone, and have been revised accordingly as suggested. We revised the description (Lines 41–43).

   "Compared to CK, intercropping reduced tea-row emissions by 7.1–7.9% but increased inter-row emissions by 12.7–28.9% based on the two years cumulative emissions, continuous intercropping significantly decreased overall inter-row emissions over time."

4. Lines 40–45: This section is unclear. Tea-row and inter-row fluxes appear similar in magnitude (e.g., 8.12 CK (tea-row) vs. 9.07 CK (inter-row)). Tea-row emissions decrease by 7–8%, while inter-row emissions increase by 13–29%. How does this support the conclusion that intercropping is a climate-smart management strategy?

   Response: Thank you for the question. This question is of great significance. In fact, the comparisons in the text should be understood within the correct frame of reference. Whether for the tea rows or the inter-rows, the emission data are relative to "tea plantations with no fertilization." For traditional agricultural economic tea plantations, the amount of fertilizer applied is huge. Most importantly, the overall emissions from the "green manure intercropping" tea plantation remain significantly lower than those from conventionally fertilized tea plantations. This demonstrates that intercropping, as an integrated system, has a clear emission reduction effect.

Furthermore, and more importantly, evidence on the temporal scale shows that over the two-year period, emissions from the tea rows continued to decrease, while emissions from the inter-rows dropped significantly in the second year compared to the first. This powerfully indicates that the emission reduction benefits of green manure intercropping require time to stabilize and fully manifest across the entire system, including the inter-rows, highlighting the critical importance of sustained implementation for achieving long-term and stable emission reductions.

5. Add to the abstract: measurement method (flux chambers), campaign duration, and dataset size.

   Response: Thank you for the comments. We revised the description (Lines 23–27).

   “We employed the static chamber method over a two-year period, with sampling conducted weekly, to investigate how intercropping with *Vulpia myuros* (SM) and a legume–nonlegume mixture of *Lolium perenne* and *Trifolium repens* (HM) influenced spatial CO_2 flux dynamics compared with a no-intercropping control (CK) from tea rows and inter-row zones in a subtropical tea plantation.**”**

Experimental Setup (Lines 164 ff.)

1. Provide more details: plantation size, distances between plots and replicates, age of plantation and tea plants, etc.

   Response: Thank you for your valuable and professional comments. The detailed information has been added to Section 2.1 (Lines 161–164) and Section 2.2 (Lines 171–175), respectively.

The added content is as follows:

In Section 2.1 (Lines 161–164):

"The tea plantation was established in 2015; the tea plants are 8–10 years old and arranged in a single-row planting pattern with a row spacing of 150 cm and a plant spacing of 40 cm."

In Section 2.2 (Lines 171–175):

"The experimental tea plantation covered an area of approximately 1000 m². For each treatment, three representative tea rows were selected as three replicates. Adjacent treatments were separated by two tea rows (~3 m), and replicate areas within the same treatment were spaced approximately 5 m apart."

2. Consider adding a schematic (with dimensions) to Figure 1. Clarify whether tea plants were inside the chambers.

Response: Thank you for your constructive comment. Figure 1b is a field photo of the experimental setup. The silver chamber shown is the flux chamber, which fully enclosed the tea plant. We have revised the figure by adding the specific dimensions of the flux chamber next to it in the image. (A photo of the chamber placed in an inter-rows location has also been added to panel b.)

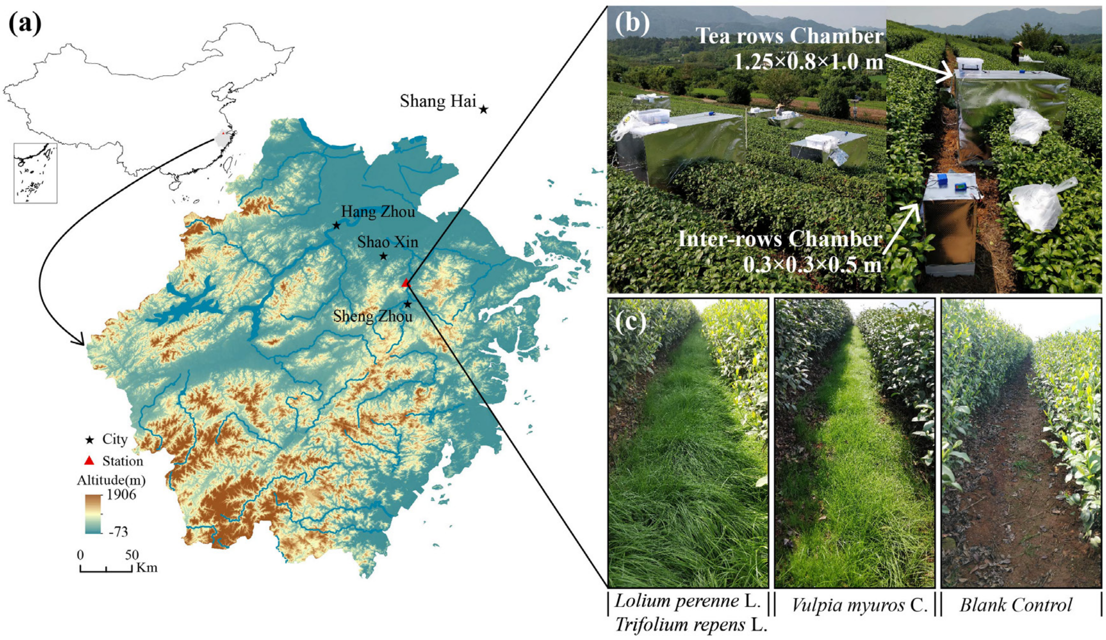


Figure 1. (a) Geographic location of the study area in Shengzhou City, Zhejiang

Province, China; (b) field layout of the tea plantation experiment; (c) photos of *Lolium perenne* L. and *Trifolium repen*s L. plot, and *Vulpia myuros* C. plot, the blank control plot, respectively.

3. According to lines 183–184, one measurement included four samples every 7 minutes: indicate when the first sample was taken after chamber closure and how long (overall) a single experiment took. How high were the CO_2 concentrations at the end?

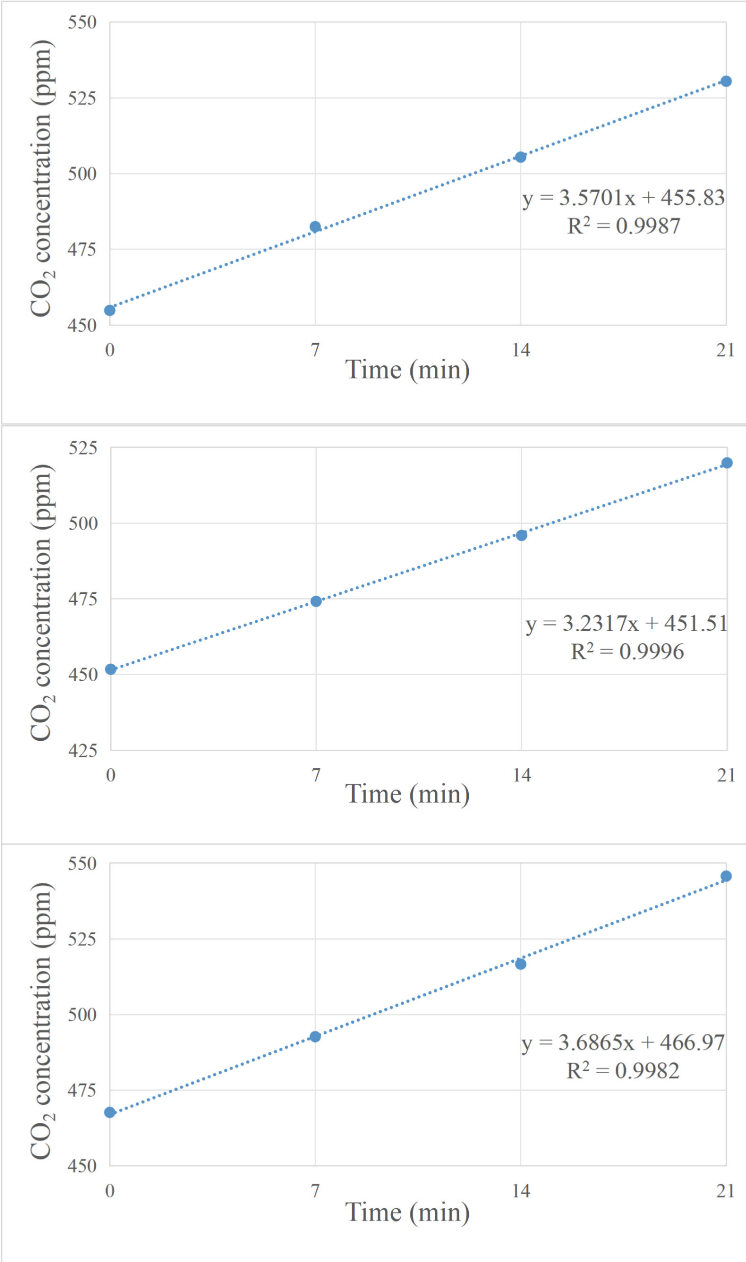
Response: Thank you for your questions. This comment covers several issues; we have addressed each of the points raised below in a point-by-point manner:

We collected four gas samples during the 21-minute period after chamber closure to calculate the flux at each point. The CO_2 concentration of the final gas sample is provided in the representative data table below. Data shown are means ± SE.

Type	Spring (ppm)	Summer (ppm)	Autumn (ppm)	Winter (ppm)
CKT	559.38±9.04	603.10±27.82	586.91±10.10	506.64±5.33f
HMT	550.06±9.72	569.38±23.78	574.68±8.07	507.70±4.94
SMT	550.44±8.61	583.63±24.70	575.51±8.85	503.52±4.85
CKG	705.94±26.25	705.93±30.60	662.62±16.00	563.34±6.88
HMG	719.34±24.49	725.38±34.82	681.12±15.92	559.16±10.55
SMG	740.50±27.08	757.70±36.72	708.70±19.18	581.16±11.75

Include an example showing the 4 measured CO_2 concentrations over time (perhaps in the appendix) and illustrate how fluxes were calculated. Was a linear fit applied to the four data points? How linear was the CO_2 increase? How consistent were the three replicates?

Response: Thank you for your questions. Fluxes were calculated by determining the linear slope from four gas concentration measurements. We added the formula in Appendix 1. All fitted slopes selected for flux calculation exhibited high linearity, with $R^2 > 0.95$. To ensure data reliability, outliers and points with large standard deviations were excluded during quality control. The resulting consistency across replicates is reflected in the standard deviations of the mean fluxes, as shown in Fig. 2b, c. The following figure illustrates the slope of the linear regression of CO_2 concentration over time.



Were all six plots always sampled simultaneously? Did you ever observe decreases in CO_2 concentrations? Figure 2 shows only positive fluxes.

Response: Thank you for your questions. Sampling was conducted sequentially across the six treatments, and each full sampling cycle was typically completed within one hour. Dark chambers covered with aluminum foil were used to measure respiratory fluxes. No significant decline in CO_2 concentration was observed during long-term flux measurements. However, a limited number of negative flux values were recorded during diurnal observations in spring and winter, as detailed in the diurnal variation section.

Include an uncertainty analysis for flux determination.

Response: Thank you for your comments. We added it (Lines 201–208)

"During the tests, the deviation between the calculated regression values of CO_2 and the nominal mole fractions was 0.37 μmol mol^{-1}. The linear fit between the instrument response values and the nominal mole fractions achieved a correlation coefficient (R^2) of 0.9999. Furthermore, the standard gases used were calibrated in multiple rounds by the Greenhouse Gas Laboratory of the Atmospheric Observation Center of the China Meteorological Administration using primary standard gases, ensuring traceability to the World Meteorological Organization primary standards."

Figure and Table Captions

1. Add a condensed explanation of your three letter codes to the different figure/table captions: Something like: CK = control, SM and HM = intercropping types, T = tea row, G = inter-row.

   Response: Thank you for this helpful suggestion. We agree that adding a condensed explanation of the letter codes to the figure and table captions will significantly improve their clarity for readers. Accordingly, we add the following note to all relevant captions: "CK for control; SM and HM for intercropping types, T for tea row, G for inter-row."

Results

1. Line 243: Explain what the uncertainties represent.

Response: The uncertainty mentioned here refers to the standard deviation. We have added a clarification regarding the flux data presentation in the text (Line 239) as follows: "Data shown are means ± standard error (SE)."

2. Line 245: Specify the significance level for "significantly higher."

   Response: Thank you for your suggestion. We have added the notation "($p < 0.05$)" in the "2.3 Data Processing" section (Lines 239–240).

   "In all statistical tests, the level of significant differences and correlations was set at $p < 0.05$."

Figures and Tables

1. Figure 2: Clarify whether error bars indicate uncertainty of a single flux measurement or standard deviation across duplicates.

   Response: Thank you for your comments. The error bars represent the standard error of the mean among repeated measurements. We have added the relevant description in the Figure 2.

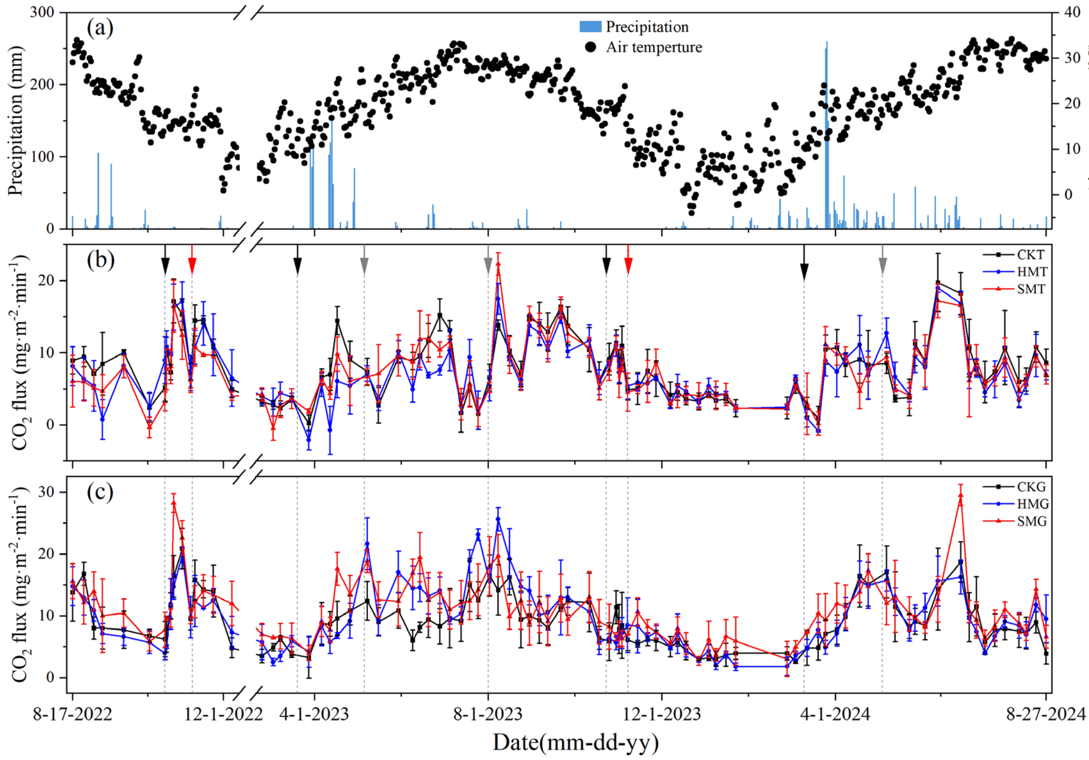
2. Table 1: Explain ± values and superscript letters.

   Response: Thank you for your comments. In this study, data are presented as mean ± standard error (SE). The use of SE is preferred when making inferences about the population mean or comparing the differences between group means. The superscript letters indicate significant differences between seasons and treatment types, as determined by two-way ANOVA. Different letters denote statistically significant differences ($p < 0.05$). we added the descriptions in Table 1.

3. Figure 2: Use a different color for precipitation vs. temperature. Confirm whether

negative CO_2 fluxes were ever observed. Were tea plants present in the chambers?

Response: Thank you for your comments. We have revised Figure 2. Measurements were predominantly conducted on clear mornings, with net CO_2 emissions observed in the majority of cases and only weak negative fluxes recorded at a few time points. The tea plant was enclosed inside the measurement chamber during sampling; it can be seen in revised Figure 1b.



4. Figure 3: Explain superscript letters and error bars. Add "T" for tea row and "G" for inter-row in the legend.

Response: Thank you for your comments. In this study, data are presented as mean ± standard error; superscript letters denote significant groupings based on two-way ANOVA with Tukey's post-hoc test. We added it (Lines 892–893).

"Data shown are means ± SE. Different superscript letters denote statistically significant differences ($p < 0.05$). CK for control; SM and HM for intercropping types, T for tea row, G for inter-row." in the Figure 3 legend.

Results

1. Lines 354 ff.: The consistent differences in soil properties are surprising given the short distances. Provide more detail on plantation history and intercropping duration. When did the intercropping practices start?

   Response: Thank you for your comments. The CKG and SMG treatments were spatially separated by three tea rows (approximately 5 m). Green manure intercropping was maintained for two years, with sowing conducted each November and decomposition occurring by August of the following year. Under the SM treatment, which involved continuous coverage of *Vulpia myuros* over two years, hence changes in soil properties were observed. The presented data represent the two-year averages of soil ammonium-N and nitrate-N concentrations. We added more detail on plantation history and intercropping duration. (Lines 161–164; 171–174; 178–180)

   "The tea plantation was established in 2015; the tea plants are 8–10 years old and arranged in a single-row planting pattern with a row spacing of 150 cm and a plant spacing of 40 cm."

   "The experimental tea plantation covered an area of approximately 1000 m². For each treatment, three representative tea rows were selected as three replicates. Adjacent treatments were separated by two tea rows (~3 m), and replicate areas within the same treatment were spaced approximately 5 m apart."

   "Basal fertilization and tillage were conducted in middle of October, followed by green manure sowing in early November."

Conclusions

1. Line 587 refers to the mitigation of carbon losses. Quantify overall CO_2 emission reductions when summing tea-row and inter-row fluxes for HM, SM, and CK. Data (e.g. in abstract, see my comment there) do not support it and Figure 3 suggest reductions only for HM in the second year. If mitigation exists, contextualize its

potential impact at larger scales.

Response: Thank you for your comments. The values mentioned are relative to the CK treatment, and we have added "compared to the CK" in line 602. We also recognize that the net mitigation effect at the small plot scale over a two-year period may be limited. The mitigation effect is primarily manifested as a significant reduction in emissions from the tea-row zone, which offsets the initial increase observed in the inter-row area. The clear declining trend in inter-row emissions under the HM treatment in the second year suggests that the initial emission pulse was likely a transient response to soil disturbance during the green-manure establishment phase, and that its mitigation potential strengthens over time. Future management could be optimized to maximize emission reduction in tea rows while minimizing disturbance in inter-row areas, thereby enhancing the overall net benefit.

2. Add general considerations on intercropping: benefits and drawbacks (costs, labor, temperature effects, soil compaction, aeration, etc.). Large-scale adoption depends on whether benefits outweigh additional costs.

   Response: We thank the reviewer for raising this important perspective. We agree that the widespread adoption of any agricultural technology depends on its net benefits. We revised the description (Lines 611–620)

   "Continuous green manure intercropping over two years significantly reduced inter-row CO_2 emissions, and the HM treatment suppressed fertilization-induced emission peaks. These findings demonstrate that green manure intercropping, particularly mixed legume and non-legume combinations, can effectively alter the spatial pattern of CO_2 emissions and mitigate carbon losses. Moreover, this practice provides significant co-benefits like improved soil aeration and fertility, reduced chemical fertilizers, weed suppression, and promoted tea plant growth, thereby offsetting the extra costs in labor and seeds. Therefore, green manure intercropping emerges as a practical and multifunctional strategy for reducing carbon emissions in agroecosystems."

Spatially Contrasting CO_2 Dynamics Driven by Green Manure Intercropping in Subtropical Tea Plantations

Shuo Liu[1,4], Zeping Jin[1,3], Ziyi Chen[1,3], Haolin Li[1,3], Zihan Fan[3], Shaohui Li[3], Haiwang Fu[1,4], Wei He[1], Kunpeng Zang[1], Shuangxi Fang[1,5*], Peng Yan[2]

[1] Zhejiang Carbon Neutral Innovation Institute & Zhejiang International Cooperation Base for Science and Technology on Carbon Emission Reduction and Monitoring, Zhejiang University of Technology, Hangzhou 310014, China

[2] Key Laboratory of Tea Quality and Safety Control, Ministry of Agriculture, Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

[3] College of Environment, Zhejiang University of Technology, Hangzhou 310014, China

[4] Shaoxing Research Institute, Zhejiang University of Technology, Shaoxing 312077, China

[5] State Key Laboratory of Green Chemical Synthesis and Conversion, Zhejiang University of Technology, Hangzhou 310014, China

Correspondence authors:

Shuangxi Fang, E-mail: fangsx@zjut.edu.cn

Abstract:

Tea plantations are important contributors to greenhouse gas emissions due to intensive fertilization and continuous cultivation. However, the mechanisms by which green manure intercropping regulates soil CO_2 dynamics in these systems remain poorly understood. We employed the static chamber method over a two-year period, with sampling conducted weekly, to investigate ~~We investigated~~ how intercropping with *Vulpia myuros* (SM) and a legume–nonlegume mixture of *Lolium perenne* and *Trifolium repens* (HM) influenced spatial CO_2 flux dynamics compared with a no-intercropping control (CK) from tea rows and inter-row zones in a subtropical tea plantation. Distinct seasonal variations were observed, with CO_2 fluxes peaking in summer and autumn and declining in spring and winter. ~~Average tea-row fluxes were 7.41 ± 0.45, 7.35 ± 0.44, and 8.12 ± 0.46 mg·m^{-2}·min^{-1} under SM, HM, and CK, respectively, indicating emission reductions with intercropping. In contrast, inter-row fluxes were higher under SM (10.83 ± 0.52 mg m^{-2} min^{-1}) and HM (9.77 ± 0.54 mg m^{-2} min^{-1}) than under CK (9.07 ± 0.44 mg m^{-2} min^{-1}), demonstrating pronounced spatial contrasts.~~ Average tea-row fluxes were 8.7% and 9.5% lower under SM and HM, respectively, compared to CK, indicating emission reductions with intercropping. In contrast, average inter-row fluxes increased by 19.4% under SM and 7.7% under HM, demonstrating pronounced spatial contrasts. ~~Diurnal patterns exhibited midday peaks (12:00–14:00), especially in spring and summer, and short-term CO_2 pulses were triggered by field operations such as fertilization and pruning.~~ Diurnal patterns generally exhibited midday peaks (12:00–14:00), especially in summer and autumn across all tea-rows, and short-term CO_2 pulses were triggered by field operations such as fertilization and pruning. Notably, HM effectively suppressed fertilization-induced CO_2 pulses, revealing the mitigation potential of legume–nonlegume mixtures. Green manure increased soil organic carbon (6.4%), lowered soil temperature (4.5%), and enhanced porosity (4.2%), collectively shaping CO_2 dynamics. Multivariate analysis identified soil organic carbon (SOC) and temperature as dominant flux drivers, and a potential SOC threshold was detected, beyond which CO_2 emissions accelerated. Compared to CK, ~~While~~ intercropping reduced tea-row emissions by 7.1–7.9% but increased inter-row emissions by 12.7–

28.9%; based on the two years cumulative emissions, continuous intercropping significantly decreased overall inter-row emissions over time. These results highlight the spatially heterogeneous nature of carbon flux regulation and demonstrate the long-term potential of green manure intercropping as a climate-smart management strategy in perennial agroecosystems.

Keywords: tea plantations, green manure, CO_2 emissions, soil factors

1 Introduction

Mitigating greenhouse gas (GHG) emissions to address global warming and associated climate challenges remains a priority in global environmental research. Among long-lived GHGs, carbon dioxide (CO_2) plays the most prominent role, contributing approximately 66% to the increase in global radiative forcing (IPCC, 2022). In 2023, the global average atmospheric CO_2 concentration reached 420.0 ± 0.1 ppm, representing a 151% increase compared to pre-industrial levels (prior to 1750) (WMO, 2024). Agriculture is a major emission sector, accounting for about 14% of total anthropogenic CO_2 emissions (Wang et al., 2025). In China, this share is even higher, with agricultural activities accounting for up to 17% of national CO_2 emissions (Xu and Lin, 2017). Therefore, accurately characterizing CO_2 emission dynamics in agricultural systems and scientifically informed mitigation strategies are critical for advancing global GHG reduction efforts and promoting sustainable, low-carbon agricultural development (Xu et al., 2024).

Tea (*Camellia sinensis* L.) is an important economic crop in tropical and subtropical regions. Over recent decades, global tea cultivation area has expanded rapidly, reaching 4.70 million hectares in 2022. China has led the most significant growth, with 3.35 million hectares of tea plantations and an annual production of 2.82 million tons, ranking first worldwide in both area and output (FAO, 2024). To maximize yield and improve tea quality, fertilizer inputs to tea cultivation area can be up to four times higher than those applied to staple crops during a single growing season (Zou et al., 2009; Han et al., 2013; Yao et al., 2015). In China, average annual fertilizer use in tea plantations reaches 678 kg ha^{-1}, with more than 30% of plantations experiencing over-application (Ni et al., 2019). Such intensive fertilization not only accelerates soil acidification but also significantly increases GHGs emissions from tea plantations (Liu et al., 2016; Yan et al., 2020). However, most existing studies on agricultural CO_2 emissions have focused on staple cropping systems such as wheat (Song et al., 2024), rice (Qian et al., 2023), and maize (Zhang et al., 2020), while studies on CO_2 emissions from tea plantations remain limited.

For instance, Lang et al. (2017) reported that intercropping rubber trees with tea in the tropical forests of Xishuangbanna, China, reduced CO_2 emissions, although it simultaneously weakened the soil's methane (CH_4) uptake capacity. Wanyama et al. (2019) found that converting tropical montane forests into tea plantations in Africa decreased annual soil CO_2 emissions to 5.6 t ha^{-1}, with emissions positively correlated with soil pH and negatively correlated with the soil C/N ratio. Pang et al. (2019) quantitatively assessed the net ecosystem exchange (NEE) of tea plantations in southeastern China from 2014 to 2017, reporting values ranging from −182.40 to −301.51 g C m^{-2}, indicating that tea plantations act as net carbon sinks. However, their carbon sequestration potential was lower than that of other subtropical ecosystems, with temperature identified as the primary factor influencing ecosystem respiration. These findings suggest that CO_2 emissions from tea plantations play a non-negligible role on the carbon exchange between atmosphere and tea plantations. However, the limited number of studies has led to substantial uncertainty on estimating tea plantation CO_2 emissions, restricting our understanding of their contribution to regional and global agricultural GHG budgets (Li et al., 2016; Ji et al., 2020) and hindering the development of low-carbon tea plantations.

In response, there is a growing emphasis on the development of eco-friendly and low-carbon tea plantations (Wang et al., 2022). Toward reduction of fertilizer usage and higher economic efficiency, various management practices were incorporated, including by using green manure. As a modernized agricultural practice, green manure has been widely adopted in farming systems and serves as an important measure for improving soil quality, playing a vital role in sustainable agriculture. Within the context of GHGs mitigation, green manure is recognized as an effective solution for improving soil quality and enhancing CO_2 sequestration in agroecosystems (Forte et al., 2017). However, most studies examining the relationship between green manure and carbon emissions have focused on conventional croplands such as rice and wheat. Comprehensive studies have shown that appropriate green manure management can significantly reduce the global warming potential (GWP) associated with fertilization (Zhang et al., 2024). For instance, Gong et al. (2021) demonstrated that long-term ryegrass cover in organic soybean fields effectively reduced net GWP. In contrast, other studies have reported that green manure application may increase CO_2 emissions. Kim et al. (2013) found that the application of Chinese milk vetch and ryegrass increased winter CO_2 fluxes in paddy fields by approximately 197% and 266%, respectively. Large-scale assessments have further revealed that green manure tends to increase CO_2 emissions, primarily due to differences in plant species and biomass inputs. Biomass alone explained 63% of the variation in CO_2 emission increases, with emissions declining as the C/N ratio of cover crop biomass increased. Notably, mixed sowing of leguminous and non-leguminous green manures has been shown to improve residue C/N ratios and reduce GHGs emissions (Muhammad et al., 2019).

Existing studies on GHG mitigation in tea systems have predominantly focused on fertilizer reduction and substitution strategies. Wu et al. (2018) conducted a three-year fertilization control experiment in southern China and found that halving nitrogen input decreased nitrous oxide (N_2O) emissions by 44.5% in tea plantations. In contrast, Yao et al. (2015) reported that organic fertilizer application led to a 71% increase in N_2O emissions compared to conventional urea, suggesting potential trade-offs in GHG outcomes. Organic amendments, such as compost or manure, have been shown to improve soil fertility, enhance soil structure, porosity, and pH, and promote carbon sequestration in tea plantation soils (Han et al., 2013; Wu et al., 2021). Biochar application has also been identified as an effective strategy for improving soil quality while simultaneously enhancing soil carbon storage and reducing emissions (Wu et al., 2021). The effect of green manure intercropping on tea plantations was mainly focused on improvements in tea plant growth and soil nutrient dynamics. For example, intercropping with green manure species has been shown to enhance nitrogen use efficiency and increase soil microbial diversity (Huang et al., 2023). The potential role of green manure intercropping in mitigating GHGs emissions in tea ecosystems remains poorly understood, and its interactions with key environmental factors have not been fully clarified (Zhu et al., 2018). Green manure may influence CO_2 emissions by altering carbon input levels and inducing soil disturbances, but the specific emission characteristics and driving factors require further investigation.

To address these gaps, this study selected cultivated tea plantations region located in the east of China, where is recognized as a very important tea cultivation area famous by the tea name of Longjing. Commonly used green manure species in tea systems (*Vulpia myuros* C., *Lolium perenne* L., and *Trifolium repens* L.) were selected for intercropping, covering both leguminous and non-leguminous species, under monoculture and mixed-sowing configurations. CO_2 flux were carried out in both tea rows and inter-row zones across different intercropping treatments. Dynamics and key influencing factors of CO_2 emissions under various green manure intercropping models were analyzed, aiming to reveal the potential reasons by which green manure intercropping regulates carbon fluxes in tea plantations and provide support for the development of low-carbon tea plantations and sustainable regional agriculture.

2 Methodology

2.1 Monitoring Site

This study was conducted at the Comprehensive Experimental Tea Plantation Base of the Tea Research Institute, Chinese Academy of Agricultural Sciences, located in Shengzhou, Zhejiang Province, China (120°83′E, 29°75′N; elevation 30 m a.s.l.) (Fig. 1a). The site is situated in a low mountainous and hilly region of southeastern China and is characterized by a subtropical monsoon climate. During the experimental period (August 2022 to August 2024), the average annual temperature was approximately 16 °C, with an average annual precipitation of about 1400 mm. The region experiences a concentrated rainy season from April to June and has a frost-free period of around 240 days. The tea cultivated at the site is *Jinmudan*, an elite cultivar derived from the hybridization of *Tieguanyin* and *Huangdan*, and is widely planted across China. The tea plantation was established in 2015;, the tea plants are 8–10 years old and arranged in a single-row planting pattern with a row spacing of 150 cm and a plant spacing of 40 cm.~~The soil type of the tea plantation is classified as red soil (Ultisol), typical soil species in this region.~~ The soil type of the tea plantation is classified as red soil (Ultisol), a typical soil type in this region, and the initial basic physicochemical properties of the six treatment soils are shown in Table 1.

2.2 Experimental Setup

Three green manure intercropping treatments were established in this study: *Vulpia myuros* C. (SM), a mixture of *Lolium perenne* L. and *Trifolium repens* L. (HM), and a control treatment without intercropping (CK). The experimental tea plantation covered an area of approximately 1000 m². For each treatment, three representative tea rows were selected as three replicates. Adjacent treatments were separated by two tea rows (~3 m), and replicate areas within the same treatment were spaced approximately 5 m apart. ~~Each treatment has three duplications.~~ Gas fluxes were measured in both tea rows (T) and inter-row zones (G), resulting in six treatments: SMT, SMG, HMT, HMG, CKT, and CKG, with a total of 18 representative sampling points (Fig. 1b, c). Tea plantation followed standard management practices, including fertilization, pruning, and tillage. Basal fertilization and tillage were conducted in middle of October, followed by green manure sowing in early November. ~~On October 21, 2022, rapeseed cake was applied as base fertilizer at a rate of 2250 kg ha^{-1} after trenching and soil turning between the tea rows. On October 28, 2023, base fertilizer consisting of rapeseed cake (975 kg ha^{-1}) and compound fertilizer (375 kg ha^{-1}) was applied following trenching and soil turning in the tea row inter-rows.~~ Urea was applied as a topdressing in early February. Tea leaves were harvested in end of March, and pruning was normally conducted in May and July.

Gas sampling was performed using the static chamber–gas chromatography method. The dimensions of the static chambers were 1.25 × 0.8 × 1.0 m for tea rows and 0.3 × 0.3 × 0.5 m for inter-row zones (Fig. 1b). Each chamber was equipped with an internal fan to ensure uniform gas mixing. To avoid rapid heating due to sunlight, the chambers were wrapped with aluminum foil and sponge, functioning as dark chambers. To minimize disturbance, chamber bases with water grooves were installed one month in advance at each sampling point, inserted 15 cm into the soil. During sampling, water was added to the grooves, and the chamber was securely sealed onto the base to create a closed environment. Four gas samples were collected at 7-minute intervals using gas sampling bags.

For seasonal monitoring, sampling was conducted once per week between 09:00 and 11:00 a.m. (local time). Intensive sampling was also carried out following key management events such as fertilization and pruning. Diurnal variation was monitored over three consecutive days in January, April, July, and October, representing winter, spring, summer, and autumn, respectively. During these campaigns, gas samples were collected every 2 hours over a 24-hour period. All gas samples were analyzed within 24 hours by using a gas chromatograph (Agilent 7890B, Agilent Inc., USA). CO_2 concentrations were measured using a flame ionization detector (FID) at a working temperature of 175 °C. High-purity nitrogen was used as the carrier gas, with an injection volume of ~~30 mL~~ 30 mL and a flow rate of ~~250 mL~~250 mL·min^{-1}. During the tests, the deviation between the calculated regression values of CO_2 and the nominal mole fractions was 0.37 μmol mol^{-1}. The linear fit between the instrument response values and the nominal mole fractions achieved a correlation coefficient (R^2) of 0.9999. Furthermore, the standard gases used were calibrated in multiple rounds by the Greenhouse Gas Laboratory of the Atmospheric Observation Center of the China Meteorological Administration using primary standard gases, ensuring traceability to the World Meteorological Organization primary standards.

Meteorological data, including precipitation, atmospheric pressure, and air temperature (AT), were obtained from an automatic weather station installed within the tea plantation. Soil samples were recorded using an automatic weather station installed within the tea plantation. Soil samples were collected monthly using a five-point composite method within a 1 m radius of each sampling point. After passing through a 2 mm sieve, the samples were divided into three portions:

i. One fresh portion was analyzed for microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) using the chloroform fumigation-extraction method and a TOC analyzer.

ii. A second portion was air-dried and ground for analysis of soil pH.

iii. The third portion was stored at 4 °C for analysis of nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) by spectrophotometry, total carbon (TC) and total nitrogen (TN) by elemental analysis, and soil organic carbon (SOC) by the dichromate oxidation–spectrophotometry method.

Soil temperature (ST) and volumetric water content (VWC) were measured *in-situ* using a portable soil sensor (TDR-315H, Acclima). Soil bulk density (BD) and water-filled pore space (WFPS) were determined using the core ring method.

2.3 Data Processing

The flux refers to the amount of gas exchanged per unit time and unit area. A positive value indicates net emission to the atmosphere, while a negative value indicates net uptake from the atmosphere (Yao et al., 2015; Zhang et al., 2020). Based on the flux measurements, cumulative CO_2 emissions under different green manure intercropping treatments were also estimated. All data analyses and visualizations were performed using R software. Two-way analysis of variance (Two-way ANOVA) was employed to assess the effects of treatment type and observation period on CO_2 fluxes and soil physicochemical properties. Spearman correlation analysis and Mantel tests were used to examine the relationships between CO_2 flux and environmental variables under different green manure intercropping treatments. Canonical correspondence analysis (CCA) was applied to comprehensively evaluate the influence of soil physicochemical properties on CO_2 emissions. Data shown are means ± standard error (SE). In all statistical tests, the level of significant differences and correlations was set at $p < 0.05$.

3 Results

3.1 Long-term Variation of CO_2 Fluxes under Green Manure Intercropping

Figure 2 illustrates the long-term trends of key environmental variables and CO_2 fluxes in the tea plantation throughout the observation period. Overall, CO_2 fluxes from both tea-row and inter-row zones displayed distinct seasonal patterns: higher in summer and autumn, and lower in spring and winter. The seasonal differences between the warm (summer and autumn) and cool (spring and winter) periods were statistically significant (Table ~~1~~2). The temporal dynamics of CO_2 fluxes closely tracked the trends in air temperature (Fig. 2a), suggesting that temperature is a key driver of soil respiration in tea plantations. Annual fluctuations in CO_2 fluxes were also strongly influenced by field management activities. For example, a sharp increase in CO_2 emissions was observed following basal fertilization in October, and another rise occurred in March of the following year after topdressing and with the onset of warmer temperatures, ultimately peaking in summer (Fig. 2b-c). The effects of management activities are further detailed in Section 3.3.

In tea rows, the annual mean CO_2 fluxes under HMT and SMT treatments were 7.35 ± 0.44 mg·m^{-2}·min^{-1} and 7.41 ± 0.45 mg·m^{-2}·min^{-1}, respectively, both lower than that of the control (CKT: 8.12 ± 0.46 mg·m^{-2}·min^{-1}) (Fig. 2b). In contrast, in inter-row zones, the annual mean CO_2 fluxes were significantly higher under HMG (9.77 ± 0.54 mg·m^{-2}·min^{-1}) and SMG (10.83 ± 0.52 mg·m^{-2}·min^{-1}) compared to the control (CKG: 9.07 ± 0.44 mg·m^{-2}·min^{-1}) ($p < 0.05$) (Fig. 2c). Across seasons, CKT generally exhibited higher CO_2 fluxes than HMT and SMT, except during winter. In the inter-row zones, both HMG and SMG showed significantly higher fluxes than CKG in summer, while SMG consistently had significantly higher CO_2 emissions than both CKG and HMG during the remaining seasons ($p < 0.05$) (Table ~~1~~2).

Overall, green manure intercropping significantly increased CO_2 emissions from inter-rows, but reduced emissions in tea rows. In terms of cumulative annual emissions, HMT and SMT resulted in 3.69 kg·m^{-2} and 3.66 kg·m^{-2} of CO_2 emissions, respectively, both lower than the 3.97 kg·m^{-2} under CKT (Fig. 3). Similarly, cumulative CO_2 emissions under HMG and SMG remained consistently higher than under CKG, but they declined from 5.76 kg·m^{-2} and 6.43 kg·m^{-2} in the first year to 4.16 kg·m^{-2} and 4.92 kg·m^{-2} in the second year, respectively (Fig. 3). Two consecutive years of green manure intercropping led to a gradual reduction in CO_2 emissions from inter-rows, indicating its potential role in long-term emission mitigation in tea plantations. CO_2 emissions from inter-rows were substantially higher than those from tea rows. Compared with the control, HM and SM intercropping increased inter-row cumulative CO_2 emissions by 12.7% and 28.9%, respectively, while reducing tea-row emissions by 7.1% and 7.9% (Fig. 3a-b). Inter-row zones accounted for 52.6%, 57.3%, and 60.8% of the total annual CO_2 emissions in the CK, HM, and SM treatments, respectively (Fig. 3c-d), indicating that the inter-row emissions cannot be ignored.

3.2 Diurnal CO_2 Variations

CO_2 fluxes in the tea plantation exhibited pronounced diurnal variations across all seasons, particularly in spring and summer (Fig. 4), likely influenced by the growth stages of green manure species. In spring, CO_2 fluxes in tea rows under all treatments showed a similar diurnal trend: an initial decline followed by a rapid increase. HMT and SMT reached their minimum fluxes at 08:00 (local time), with values of –3.74 $mg \cdot m^{-2} \cdot min^{-1}$ and –3.80 $mg \cdot m^{-2} \cdot min^{-1}$, respectively, then rose sharply and stabilized in the afternoon. The diurnal amplitudes under HMT and SMT were notably greater than that of the control (CKT) (Fig. 4a). In the inter-row zones, the diurnal patterns under green manure treatments differed notably from the control (Fig. 4b). CKG displayed a unimodal pattern with a peak at 12:00 (12.74 $mg \cdot m^{-2} \cdot min^{-1}$) and a trough at 08:00 (5.45 $mg \cdot m^{-2} \cdot min^{-1}$), resulting in an amplitude of 7.29 $mg \cdot m^{-2} \cdot min^{-1}$. In contrast, HMG and SMG exhibited later peaks at 16:00 (23.26 $mg \cdot m^{-2} \cdot min^{-1}$) and 14:00 (24.17 $mg \cdot m^{-2} \cdot min^{-1}$), respectively, with troughs also at 08:00 (HMG: 12.28 $mg \cdot m^{-2} \cdot min^{-1}$; SMG: 12.43 $mg \cdot m^{-2} \cdot min^{-1}$). Both treatments showed substantially higher amplitudes than CKG.

Summer exhibited the most pronounced diurnal variation of CO_2 fluxes across all seasons. In tea rows, CKT, HMT, and SMT followed a bimodal pattern, with peaks at 02:00 and 12:00, and a trough at 08:00. Their respective diurnal amplitudes were 12.96, 6.70, and 10.10 $mg \cdot m^{-2} \cdot min^{-1}$ (Fig. 4c). In the inter-rows, the amplitudes were relatively lower, 7.72, 8.12, and 7.79 $mg \cdot m^{-2} \cdot min^{-1}$ for CKG, HMG, and SMG, respectively, indicating smaller fluctuations compared to tea rows (Fig. 4d). Notably, summer also showed the most distinct contrast between tea rows and inter-rows: CKT recorded the highest average flux in the tea rows, while CKG had the lowest in the inter-rows.

In autumn, tea-row fluxes under all treatments exhibited a unimodal pattern, with minima at 08:00 and peaks at 14:00. The diurnal amplitudes were 11.27, 8.02, and 12.75 $mg \cdot m^{-2} \cdot min^{-1}$ for CKT, HMT, and SMT, respectively (Fig. 4e). In the inter-rows, HMG and SMG displayed relatively stable diurnal trends, whereas CKG showed a bimodal pattern with peaks at 06:00 and 16:00, and a greater amplitude than both HMG and

SMG (Fig. 4f).

In winter, CO_2 fluxes showed the most stable diurnal variation of the year. In tea rows, amplitudes were only 2.96, 2.84, and 4.92 mg·m^{-2}·min^{-1} for CKT, HMT, and SMT, respectively (Fig. 4g). Unlike other seasons, 08:00 no longer corresponded to the daily minimum but rather to a relative maximum, with daily peaks generally occurring at 14:00. In inter-rows, diurnal patterns were less defined. SMG exhibited the highest flux at 08:00 (7.57 mg·m^{-2}·min^{-1}), while HMG showed the lowest at 14:00 (0.99 mg·m^{-2}·min^{-1}) (Fig. 4h).

3.3 Effect of Human Management on CO_2 Fluxes

CO_2 fluxes from the tea field varied significantly across different growth stages of green manure, exhibiting a general increasing trend from the early growth stage to the vigorous, wilting, and decomposition stages (Fig. 5a). In the tea rows, the lowest fluxes were observed during the early growth stage, while the highest occurred during the decomposition stage. Differences among the three treatments (CKT, HMT, and SMT) were minimal during the early growth but became more apparent in the subsequent stages. Notably, during the vigorous stage, both HMT and SMT treatments reduced CO_2 emissions compared to CKT. In contrast, the impact of green manure growth on CO_2 fluxes was more pronounced in the inter-row zones (Fig. 5b). At all growth stages, CO_2 fluxes under the HMG and SMG treatments were significantly higher than those under CKG, with the largest differences observed during the wilting stage. Peak emissions occurred during the decomposition stage for HMG and during the wilting stage for SMG.

Fertilization substantially increased CO_2 emissions across the tea plantation (Fig. 5c-d). In tea rows, the post-fertilization increase in CO_2 flux under HMT was 43.1% lower than that under CKT, whereas SMT showed a 9.2% higher increase. In the inter-row zones, HMG reduced the fertilization-induced increase by 10.4% compared to CKG, while SMG amplified it by 40.1%. These findings indicate that the HM treatment can effectively mitigated CO_2 emissions triggered by fertilization, while SM treatment may intensify them, revealing the potential of legume-based mixed green manure to reduce CO_2 emissions in tea plantations. It is worth noting that the mitigation effect in the inter-row zones was weaker than in the tea rows, possibly due to differences in root distribution or organic matter inputs.

The effects of grass planting and tea pruning on CO_2 fluxes varied by treatment type and location (tea row or inter-row) (Fig. 5c-d). In the CK treatment, grass planting had no significant impact on CO_2 fluxes. However, the HM treatment led to a marked increase after grass planting, with inter-row fluxes rising by 1.81 $mg \cdot m^{-2} \cdot min^{-1}$. Similarly, the SM treatment showed significant increases in both zones, with an increase of 0.90 $mg \cdot m^{-2} \cdot min^{-1}$ in tea rows and an inter-row increase that was 3.8 times greater. These increases can be attribute to soil disturbance during sowing.

After tea pruning, no significant changes in CO_2 flux were observed in the CK treatment. However, both HMT and SMT significantly increased CO_2 emissions in tea row, with increments of 2.74 $mg \cdot m^{-2} \cdot min^{-1}$ and 2.94 $mg \cdot m^{-2} \cdot min^{-1}$, respectively. In the inter-row zones, only the HMG treatment exhibited a significant post-pruning increase of 3.25 $mg \cdot m^{-2} \cdot min^{-1}$. These increases may be attributed to pruning residues covering the green manure surface, which could elevate soil temperature and moisture, thereby enhancing soil respiration and CO_2 emissions.

3.4 Effects of Environmental Factors on CO_2 Fluxes

Significant differences in soil nutrient parameters were observed between tea rows and inter-row zones under various green manure intercropping treatments (Fig. 6). Green manure treatments generally increased soil total carbon (TC) and total nitrogen (TN), with consistently higher TC and TN levels in the inter-row zones than in the tea rows (Fig. 6a-b), resulting in significantly higher C/N ratios in the tea rows (Fig. 6c). Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) concentrations were also significantly greater in the inter-row zones, with the highest NH_4^+-N found in CKG (71.20 $mg \cdot kg^{-1}$) and the highest NO_3^--N in SMG (14.56 $mg \cdot kg^{-1}$). All green manure treatments significantly increased soil organic carbon (SOC), the average SOC contents under HM and SM were 3.6% and 9.3% higher than under CK, respectively (Fig. 6f). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) showed no significant differences between tea rows and inter-row zones, but both were slightly elevated under green manure treatments (Fig. 6g-h). Soil pH ranged from 3.6 to 4.5, with no significant differences among treatments, although green manure application slightly increased soil pH (Fig. 6i).

During the monitoring, soil temperature ranged from 2.3–41.8°C in CK, 3.0–37.6°C in HM, and 3.5–36.6°C in SM. Average soil temperatures in HM and SM were 4.5% and 3.9% lower than in CK, indicating a cooling effect of green manure. Additionally, bulk density was reduced by 8.9% and 5.0% in HM and SM compared to CK, while total porosity increased by 5.3% and 3.0%, and WFPS decreased by 29.1% and 11.1%, respectively. These results suggest that green manure intercropping effectively reduces soil compaction and improves soil aeration. The combined effect of these factors is the key to the changes in CO_2 emissions.

To further clarify these relationships, we examined the correlations between CO_2 fluxes and environmental factors under different green manure treatments (Fig. 7). CO_2 fluxes across nearly all treatments were significantly positively correlated with air temperature (AT) and soil temperature (ST) ($r > 0.5$, $p < 0.05$), suggesting temperature as a major driver of CO_2 emissions in tea plantations. Treatment-specific differences were also apparent: in green manure treatments (HM and SM), both TC and SOC in tea rows and inter-row zones showed negative correlations with CO_2 flux, whereas volumetric water content (VWC) showed significant positive correlation ($r > 0.5$, $p < 0.05$). In contrast, VWC was negatively correlated with CO_2 flux under CK treatment. These findings suggest that green manure intercropping alters soil pore structure and moisture regimes, thereby modifying CO_2 emission dynamics compared to bare soil conditions. Additionally, environmental controls on CO_2 fluxes differed between tea rows and inter-row zones. Emissions in tea rows appeared less sensitive to environmental fluctuations, likely due to the moderating effects of tea canopy coverage and root systems.

Canonical correspondence analysis (CCA) was further performed to examine the effect of green manure intercropping patterns and soil properties on CO_2 emissions in tea rows and inter-row zones (Fig. 8). The first two CCA axes explained 52.79% and

11.15% of the total variance, respectively. CCA1 was primarily associated with NH_4^+-N, NO_3^--N, SOC, and the C/N ratio, while CCA2 was mainly linked to TN, TC, MBN, MBC, and pH. These results indicate distinct environmental drivers of CO_2 emissions between the two spatial zones. In tea rows, CO_2 flux was positively associated with NO_3^--N and the C/N ratio, with relatively minor influence from pH. The influencing soil factors were similar for CKT and SMT, whereas HMT displayed a distinct pattern, likely attributable to the presence of leguminous green manure. In inter-row zones, SOC emerged as the dominant factor controlling CO_2 fluxes in HMG and SMG treatments, whereas NH_4^+-N was the key driver in CKG. Moreover, TC, TN, MBC, and MBN all showed positive associations with inter-row CO_2 fluxes, with consistent soil drivers under HMG and SMG that differed from CKG, indicating that green manure significantly affects soil–CO_2 interactions.

4 Discussion

4.1 CO_2 Flux Dynamics under Green Manure Intercropping

This study revealed pronounced seasonal variations in CO_2 fluxes from tea plantations, which were closely aligned with fluctuations in air temperature (Fig. 2a-c). In spring, rising temperatures enhanced both plant and microbial respiration, leading to a sharp increase in CO_2 emissions (Yan et al., 2022). During summer, when temperatures reached their annual peak, intensified microbial activity accelerated the decomposition of soil organic matter, resulting in the highest CO_2 fluxes of the year (Allison et al., 2010). In autumn, declining temperatures and light availability reduced microbial activity and soil respiration, thereby lowering CO_2 emissions (Liu et al., 2020). In winter, low temperatures significantly inhibited both plant and microbial respiration, causing CO_2 fluxes to drop to their annual minimum (Schnecker et al., 2023). These seasonal flux patterns were consistent throughout the two-year observation period, indicating the pivotal role of temperature in regulating CO_2 emissions in tea plantations (Chen et al., 2021).

Compared with the CK treatment, HM and SM increased annual cumulative CO_2 emissions by 3.3% and 7.9%, respectively, revealing that green manure intercropping significantly elevated total CO_2 emissions. Similar findings have been reported in previous studies. For example, Lee et al. (2021) observed consistently higher cumulative CO_2 emissions in cropland soils under green manure treatments than under fallow conditions. A meta-analysis by Muhammad et al. (2019) also showed that the use of cover crops generally increases CO_2 emissions compared with bare soil. This effect can be attributed to two possible mechanisms: 1) green manure crops introduce exogenous carbon inputs into the soil, which stimulates CO_2 release (Ho et al., 2021); and 2) green manure intercropping reduces soil bulk density and increases total porosity (Song et al., 2016), thereby improving soil aeration and promoting aerobic microbial activity. The enhanced microbial activity accelerates the decomposition and mineralization of soil organic matter, consequently increasing CO_2 emissions (Chen et al., 2019). By contrast, under the CK treatment, higher WFPS and greater soil compaction may have inhibited gas diffusion and limited CO_2 release into the atmosphere (Lang et al., 2017).

Diurnal variations of CO_2 fluxes were influenced by both seasonal dynamics and the growth stages of green manure crops. CO_2 fluxes fluctuated most sharply during spring and summer, with temperature identified as the primary driver of daily flux patterns (Pang et al., 2019). In spring, negative CO_2 flux peaks were observed in tea rows under HMT and SMT around 08:00, due to low morning temperatures suppressing microbial respiration. Moreover, the presence of easily degradable organic matter from green manure may have diverted microbial metabolism toward biomass accumulation rather than complete mineralization to CO_2. In summer, distinct spatial differences appeared between tea rows and inter-row zones. In tea rows, CO_2 fluxes under the CKT treatment were significantly higher than those under HMT and SMT, while in the inter-row zones, fluxes under HMG and SMG were higher than those under CKG. This spatial heterogeneity highlights the dual role of green manure: in tea rows, the shading effect of green manure canopy reduced soil temperatures, thereby inhibiting microbial respiration; in contrast, inter-row zones were exposed to direct sunlight, root exudates and decomposing plant residues provided additional carbon sources. Under favorable thermal conditions, this stimulated microbial activity and thus increased CO_2 emissions

(Gui et al., 2024). In autumn and winter, CO_2 flux peaks were mostly recorded in the afternoon, possibly due to rising temperatures reaching a threshold that accelerated enzymatic reactions and microbial metabolism, enhancing root and soil respiration and thus elevating CO_2 emissions (Dove et al., 2021).

4.2 Influence of Cultivation Management on CO_2 Fluxes

Fertilization, pruning, and soil tillage with cover cropping are critical anthropogenic management practices in tea plantations that significantly affect CO_2 flux dynamics. Fertilizer application, in particular, is a major contributor to agricultural greenhouse gas emissions, with emission strength influenced by the type, amount, and method of application (Wang et al., 2024). In this study, the application of rapeseed cake and compound fertilizers significantly increased soil CO_2 fluxes, especially within tea rows. This increase can be attributed to two main factors: 1) the input of exogenous organic matter enriched soil organic carbon content; and 2) trench fertilization caused physical disturbance, disrupting soil aggregates and accelerating the decomposition of soil organic carbon. These disturbances stimulated the abundance and metabolic activity of aerobic heterotrophic microbes, promoting organic matter mineralization and resulting in elevated CO_2 emissions (Chappell et al., 2015; Struck et al., 2020). Intensive pruning conducted in May and August further contributed to increased CO_2 fluxes. Pruning substantially reduces the photosynthetic biomass of tea plants, diminishing their carbon sequestration capacity. Simultaneously, the resulting litterfall provides abundant substrates for microbial respiration (Pang et al., 2019). The combined effect of reduced carbon uptake and increased decomposition substrates leads to a rapid short-term increase in CO_2 emissions after pruning.

Previous studies have demonstrated that intercropping systems introduce readily decomposable carbon through root exudates, while green manure decomposition increases organic matter inputs and improves soil organic carbon storage (Gui et al., 2024). In this study, CO_2 fluxes in inter-row zones were significantly higher than the control during the wilting and decomposition stages of green manure, suggesting that microbial activity was enhanced during these periods, thereby accelerating the decomposition and transformation of organic matter and intensifying soil respiration. Additionally, soils under green manure treatments exhibited lower annual average temperatures compared to the control, indicating that intercropping with green manure moderated surface soil temperatures and reduced daily temperature fluctuations. This effect was particularly pronounced in summer, when green manure not only reduced inter-row CO_2 emissions but also improved the microclimatic conditions of the tea plantation.

Traditional CO_2 flux measurements in tea plantations have mostly focused only on tea rows, often neglecting inter-row soil emissions (Yao et al., 2015; Chen et al., 2021). In this study, static chambers were parallelly employed to measure CO_2 fluxes in tea rows and inter-row areas, enabling a more accurate understanding of CO_2 emissions. Results showed that inter-row CO_2 fluxes were significantly higher than those in tea rows ($p < 0.05$), accounting for 52.6%, 57.3%, and 60.8% of the annual cumulative CO_2 emissions under CK, HM, and SM treatments, respectively. These findings emphasize the substantial contribution of inter-row zones to overall CO_2 emissions. This discrepancy is due to differences in management intensity: fertilization and tillage are commonly performed in inter-row areas, while the soil beneath tea canopies experiences minimal disturbance (Hirono and Nonaka, 2012). Moreover, pruning residues often accumulate in inter-row zones, further intensifying microbial activity and CO_2 emissions in these areas. The cumulative CO_2 emissions under HMG and SMG treatments were significantly lower in the second year. ~~This reduction may be attributed to reduced human disturbance and the regulatory effects of green manure (Gui et al., 2024).~~ The decrease in emissions can be attributed to the gradual attenuation of the carbon priming effect induced by soil disturbance during the initial experimental phase (Zhou, 2025), coupled with the long-term positive effects of green manure on enhancing soil physical structure and ecosystem stability (Gui et al., 2024). The increase in green manure biomass in the following year indicates that the green manure system is transitioning from an initially disturbed and unstable state toward a more productive and carbon-sequestration-enhanced stable state (Figure A1). This trend not only reflects the improved functioning of the soil ecosystem but also serves as an important driver for further carbon sequestration, contributing significantly to the reduction in inter-row CO_2 emissions observed in the following year. Therefore, long-term and systematic monitoring of inter-row soil CO_2 emissions is essential for accurately assessing the carbon dynamics and mitigation potential of tea plantation ecosystems.

4.3 Differences in Environmental Drivers

Soil CO_2 fluxes are regulated by multiple environmental factors, including photosynthetic activity or vegetation productivity (Tang et al., 2005), and soil properties such as temperature and moisture (Liu et al., 2023; Widanagamage et al., 2025). Among them, temperature is widely recognized as a primary driver of seasonal variation in soil respiration (Lang et al., 2017). Our results showed that CO_2 fluxes in both tea rows and inter-row areas were significantly correlated with soil and air temperatures under different green manure treatments (Fig. 7). In addition, carbon and nitrogen transformation processes driven by microorganisms are closely coupled. Nitrification and denitrification alter NO_3^-–N and NH_4^+–N levels, thereby influencing soil physicochemical properties and microbial activity. As a result, CO_2 emissions exhibit significant positive correlations with nitrogen mineralization, denitrification, and N_2O emissions (Dai et al., 2020). This carbon–nitrogen coupling may interact with the distinctive nutrient uptake characteristics of tea plants, which are ammonium-preferring species with rapid NH_4^+ assimilation (Xin et al., 2024). In our study, CO_2 fluxes under the CKG treatment were positively correlated with NH_4^+–N content (Fig. 9). NH_4^+–N levels under this treatment (71.20 mg·kg^{-1}) were significantly higher than in the SMG and HMG treatments, whereas the corresponding soil pH value (3.97) was significantly lower ($p < 0.05$). This concurrent high NH_4^+–N level and strong acidification is due to ammonium accumulation under conventional fertilization and subsequent H^+ release during nitrification (Chen et al., 2021). By contrast, the soil pH under green manure intercropping treatments increased by 0.02–0.11 units compared to the CK treatment (Fig. 6i), suggesting that root exudates and organic matter inputs from green manure buffered soil acidification by reducing H^+ release during NH_4^+ nitrification. Moreover, the NO_3^-–N concentration in the SMG treatment (14.56 mg·kg^{-1}) was significantly higher than that in other treatments (Fig. 6e), due to the high biomass of *Vulpia myuros* C., which may reduce nitrate losses via runoff or leaching. Its active root system also improved soil aeration, inhibiting denitrification under anoxic conditions.

SOC content reflects the dynamic balance between organic matter inputs and decomposition (Mo et al., 2024). In our study, SOC levels in the HMT and SMT treatments were higher than those in the CKT treatment, while their cumulative annual CO_2 emissions were lower. This indicates that increasing SOC storage can help mitigate greenhouse gas emissions, consistent with findings by Han et al. (2022). However, the HMG and SMG treatments exhibited much higher SOC levels than CKG, while their cumulative CO_2 emissions exceeded those of CKG. This implies that once SOC accumulation surpasses a certain threshold, the excess carbon supply may stimulate microbial activity and subsequently increase CO_2 emissions (Lim and Choi, 2014). Interestingly, recent studies reveal that SOC thresholds can modulate the impact of nitrogen fertilization on carbon sequestration. In SOC-poor soils, nitrogen inputs tend to promote carbon accumulation and soil aggregation, enhancing SOC storage. Conversely, in SOC-rich soils, nitrogen fertilization may enhance microbial metabolic efficiency and increase microbial residue production (Ling et al., 2025). Studies in different climatic zones of China have revealed that SOC thresholds are influenced by factors such as climate and soil type. In the maritime monsoon climate zone, dual thresholds for NO_3^--N and extractable iron (Fe) have been identified, beyond which their marginal effects on SOC shift significantly. In the continental monsoon climate zone, SOC content increases markedly once a critical threshold of TN is exceeded (Cui, 2025). Additionally, research in alpine ecosystems has shown that SOC components vary along elevation gradients and exhibit distinct thresholds (Zhang, 2025). These insights provide a new perspective for interpreting our results and highlight the importance of identifying threshold values under multifactorial interactions to better assess their effects on CO_2 emissions.

In tea rows, excessively high soil C/N ratios may result in nitrogen limitation, thereby inhibiting rapid decomposition of organic matter and reducing CO_2 fluxes.

Green manure, as a fresh plant residue with a relatively low C/N ratio, can be rapidly decomposed by soil microbes after incorporation, thus maintaining or enhancing SOC levels (Li et al., 2024), which aligns with our observations (Fig. 7d). MBC and MBN are generally considered closely linked to SOC (Gao et al., 2022). However, in our study, a significant positive correlation between MBC and SOC was only observed in the CKT treatment. The lack of correlation under green manure treatments may be due to the rapid and excessive input of exogenous carbon, which complicates the relationship between these variables. No significant differences in MBC and MBN levels were found among green manure treatments, and both showed weak correlations with CO_2 fluxes (Fig. 8), suggesting that MBC and MBN are not key drivers of CO_2 emissions in tea plantations.

5 Conclusion

This study revealed the regulation of CO_2 fluxes in tea plantations under different green manure intercropping treatments. Green manure significantly influenced CO_2 flux dynamics, with pronounced seasonal variations, higher fluxes in summer and autumn and lower fluxes in spring and winter. CO_2 emissions from inter-row areas were consistently higher than those from tea rows. CO_2 fluxes in the SM and HM treatments were significantly lower than in the CK treatment within tea rows, while the opposite trend was observed in inter-row areas, suggesting distinct spatial responses to green manure intercropping. Over the observation period, the HM and SM treatments reduced CO_2 emissions from tea rows by 7.1%–7.9% compared to the CK, while increasing inter-row emissions by 12.7%–28.9%. Inter-row CO_2 emissions accounted for 52.6%, 57.3%, and 60.8% of the annual cumulative CO_2 fluxes under the CK, HM, and SM treatments, respectively. These findings highlight the importance of incorporating spatial emission weighting into carbon accounting for agricultural ecosystems.

The observed spatial differences in CO_2 fluxes were closely related to variations in SOC content. Our findings suggest the existence of a critical SOC threshold that determines whether CO_2 emissions increase or decrease. Future research should focus on quantifying such thresholds under multi-factor interactions to better assess their impacts on greenhouse gas dynamics. Continuous green manure intercropping over two years significantly mitigated CO_2 emissions from inter-row areas. The HM treatment exhibited distinct diurnal CO_2 flux patterns and effectively suppressed fertilization-induced CO_2 emissions. These results demonstrate that green manure intercropping, particularly mixed legume and non-legume combinations, not only modifies the spatial pattern of CO_2 emissions in tea plantations but also provides a practical strategy for mitigating carbon losses from managed agroecosystems.Continuous green manure intercropping over two years significantly reduced inter-row CO_2 emissions, and the HM treatment suppressed fertilization-induced emission peaks. These findings demonstrate that green manure intercropping, particularly mixed legume and non-legume combinations, can effectively alter the spatial pattern of CO_2 emissions and mitigate carbon losses. Moreover, this practice provides significant co-benefits like improved soil aeration and fertility, reduced chemical fertilizers, weed suppression, and promoted tea plant growth, thereby offsetting the extra costs in labor and seeds. Therefore, green manure intercropping emerges as a practical and multifunctional strategy for reducing carbon emissions in agroecosystems.Moreover, this practice provides multiple co-benefits, such as improving soil aeration, enhancing soil fertility to reduce chemical fertilizer use, suppressing weeds, and promoting tea plant growth—factors that can economically offset the additional labor and seed costs. In summary, green manure intercropping offers a viable strategy for carbon emission reduction in agricultural ecosystems.

Data availability

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

Author contributions

S. Liu conceived and designed the study, performed the data analysis, and drafted the manuscript. S. Liu, Z. Jin, and Z. Chen contributed to data visualization. H. Li, Z. Fan, S. Li, H. Fu, K. Zang, W. He, and P. Yan conducted field and laboratory work. S. Fang supervised the research, provided funding, and contributed to manuscript review and editing.

Competing interests

The contact author has declared that none of the authors has any competing interests.

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Tables and Figures

Table 1. Initial basic physicochemical properties of the six ~~treatment~~treatments.

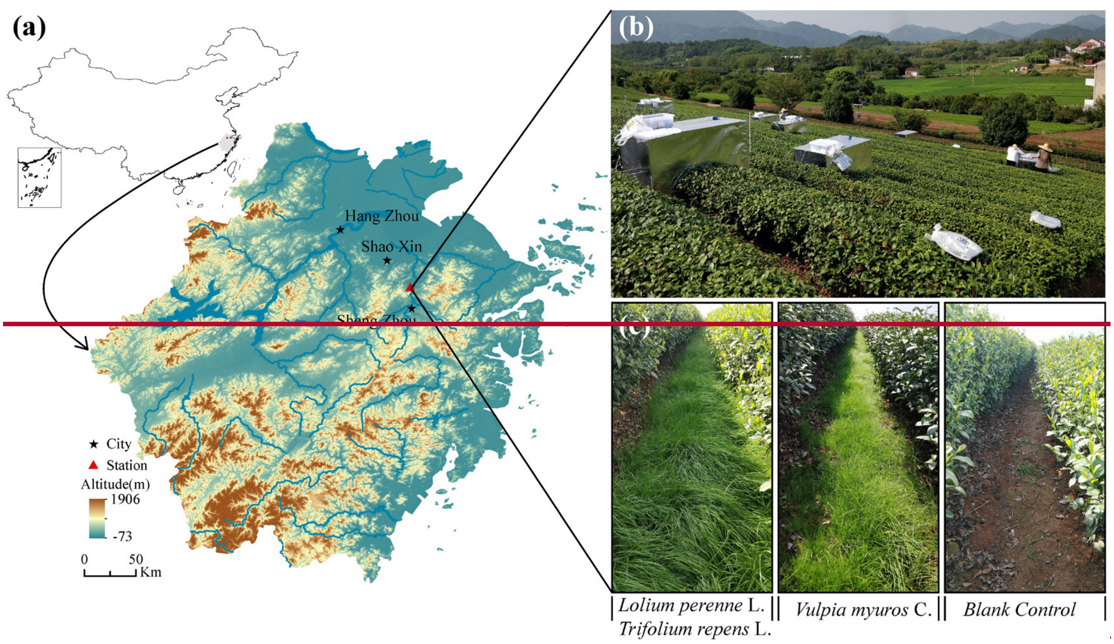
Tpye	pH	NH_4^+–N ($mg \cdot kg^{-1}$)	NO_3^-–N ($mg \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	TC ($g \cdot kg^{-1}$)	SOC ($g \cdot kg^{-1}$)
CKT	4.25±0.06^{a}	29.67±7.50^{b}	5.01±2.02^{b}	2.16±0.16^{ab}	22.93±2.00^{a}	25.50±2.65^{a}
HMT	4.01±0.06^{ab}	37.33±4.43^{ab}	6.32±2.25^{b}	2.01±0.09^{ab}	21.08±1.12^{a}	22.00±1.69^{a}
SMT	4.11±0.06^{abc}	30.42±8.93b	5.10±1.88^{b}	1.96±0.12^{b}	20.47±1.12^{a}	21.83±1.92^{a}
CKG	4.16±0.02^{ab}	33.20±5.90^{ab}	8.59±1.46^{ab}	2.41±0.19^{ab}	25.70±1.99^{a}	21.68±1.82^{a}
HMG	3.98±0.05^{b}	37.90±7.98^{ab}	12.73±3.94^{a}	2.70±0.35^{a}	27.77±3.57^{a}	27.98±3.55^{a}
SMG	4.00±0.03^{b}	44.40±7.33^{a}	13.36±3.82^{a}	2.31±0.28^{ab}	24.92±2.86^{a}	26.88±2.53^{a}

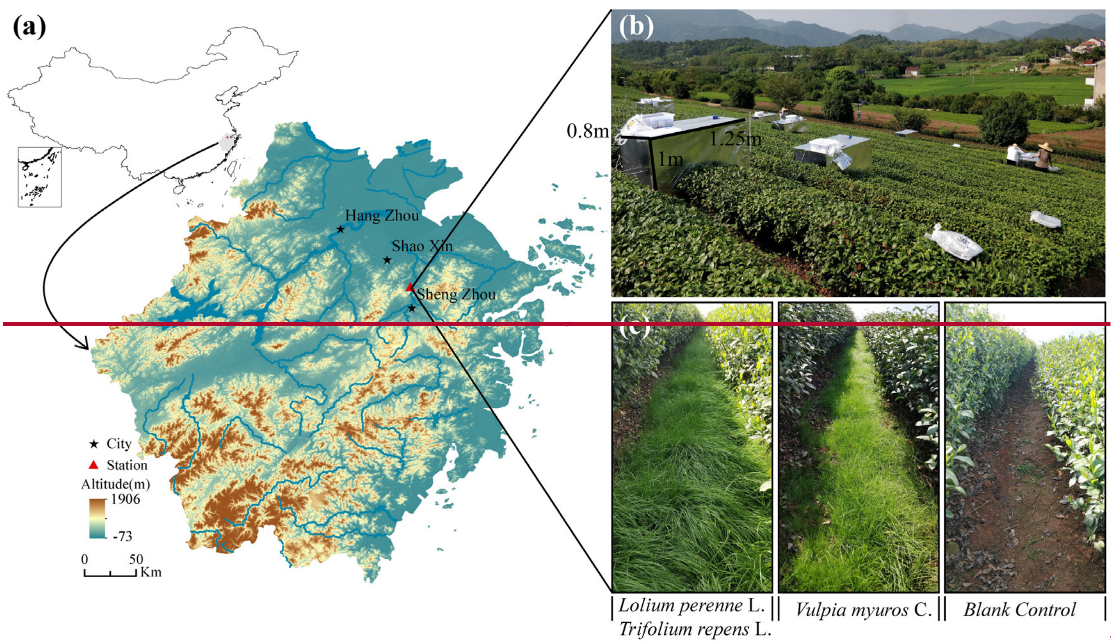
*Data shown are means ± SE. Different superscript letters indicate the significant difference ($p < 0.05$). CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

Table ~~1~~2. Seasonal variation in CO_2 fluxes from tea rows and inter-row zones under different green manure intercropping treatments. ~~Different lowercase letters indicate significant differences among treatments and seasons (*P* < 0.05).~~

Type	Spring (mg·m^{-2}·min^{-1})	Summer (mg·m^{-2}·min^{-1})	Autumn (mg·m^{-2}·min^{-1})	Winter (mg·m^{-2}·min^{-1})
CKT	6.46±0.58^{bcd}	9.66±0.49^{a}	9.93±0.68^{a}	3.60±0.36^{f}
HMT	5.65±0.44^{def}	7.97±0.39^{abc}	9.21±0.46^{a}	4.10±0.43^{ef}
SMT	6.05±0.39^{cde}	8.99±0.34^{a}	8.47±0.30^{ab}	3.71±0.37^{f}
CKG	8.83±1.14^{cd}	10.63±0.66^{abc}	9.76±0.83^{ab}	4.03±0.52^{e}
HMG	9.08±0.49^{cd}	13.03±0.29^{a}	9.38±0.39^{bcd}	4.19±0.29^{e}
SMG	10.76±0.43^{ab}	12.69±0.73^{ab}	10.94±0.86^{abc}	6.03±0.84^{de}

*Different superscript~~lowercase~~ letters indicate significant differences among treatments and seasons (*p* < 0.05). Data shown are means ± SE. CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.


(a)
Hang Zhou
Shao Xin
Sheng Zhou
City
Station
Altitude(m)
1906
-73
0
50
Km
(b)
(c)
Lolium perenne L.
Trifolium repens L.
Vulpia myuros C.
Blank Control


(a)
Hang Zhou
Shao Xin
Sheng Zhou
City
Station
Altitude(m)
1906
-73
0
50
Km
(b)
0.8m
1.25m
1m
(c)
Lolium perenne L.
Trifolium repens L.
Vulpia myuros C.
Blank Control

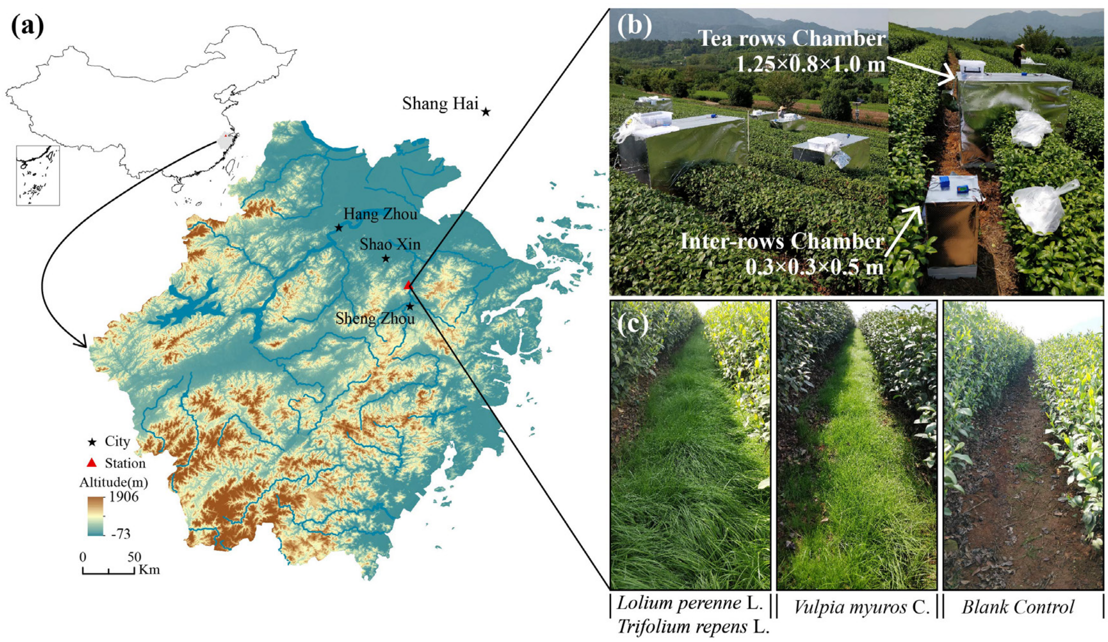
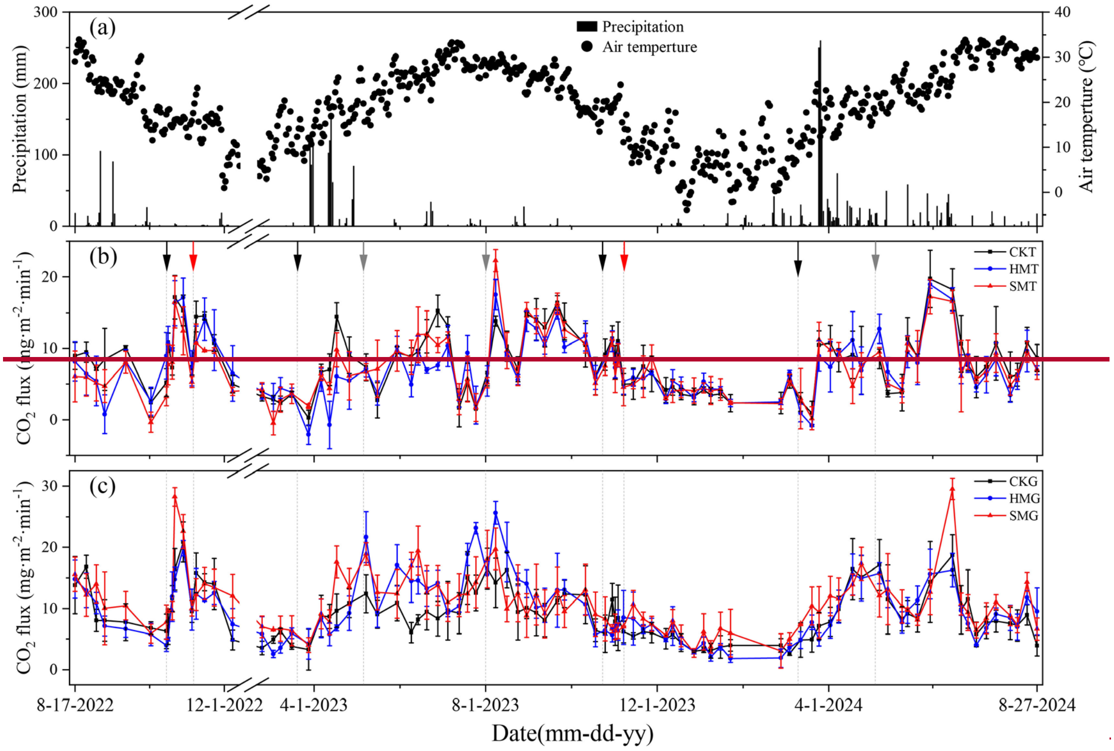


Figure 1. (a) Geographic location of the study area in Shengzhou City, Zhejiang Province, China; (b) field layout of the tea plantation experiment; (c) photos of *Lolium perenne* L. and *Trifolium repen*s L. plot, and *Vulpia myuros* C. plot, the blank~~are~~ control plot, respectively.



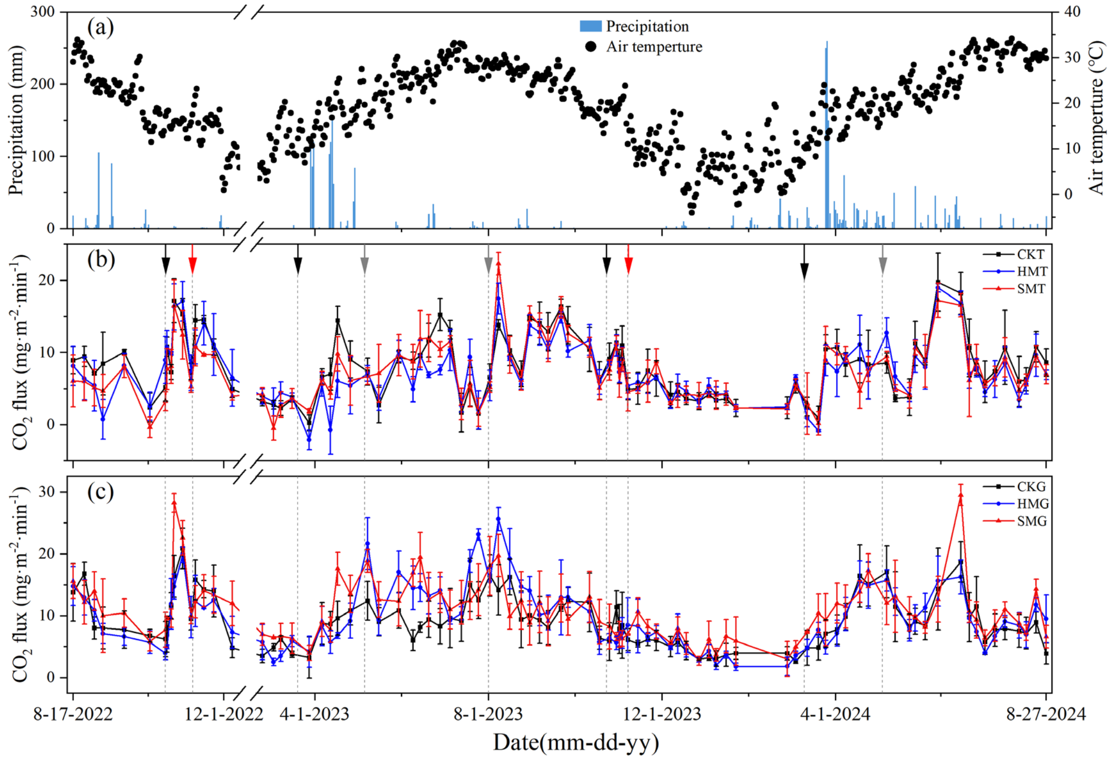


Figure 2. Dynamics of (a) air temperature and precipitation, (b) CO_2 fluxes from tea rows, and (c) CO_2 fluxes from inter-rows during the observation period (2022–2024). Black, green and orange arrows represent the timings of fertilization, grass planting and tea pruning, respectively. Flux data are presented as mean ± SE. CK for control; SM

and HM for intercropping types, T for tea row, G for inter-row.

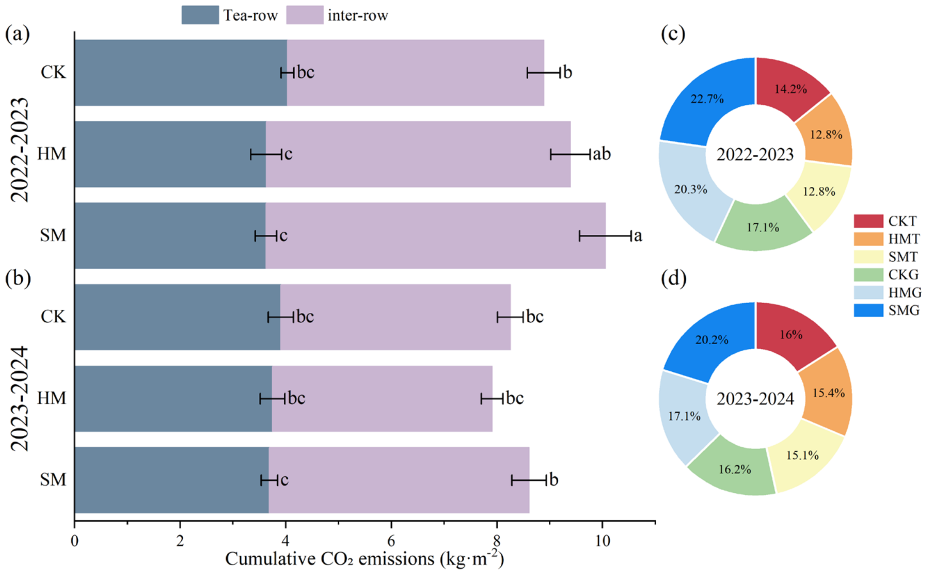


Figure 3. (a, b) Annual cumulative CO_2 emissions from tea rows and inter-rows under different green manure intercropping treatments; (c, d) contribution of tea rows and inter-rows to total annual CO_2 emissions under each treatment. Data shown are means ± SE. Different superscript letters denote statistically significant differences ($p < 0.05$). CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

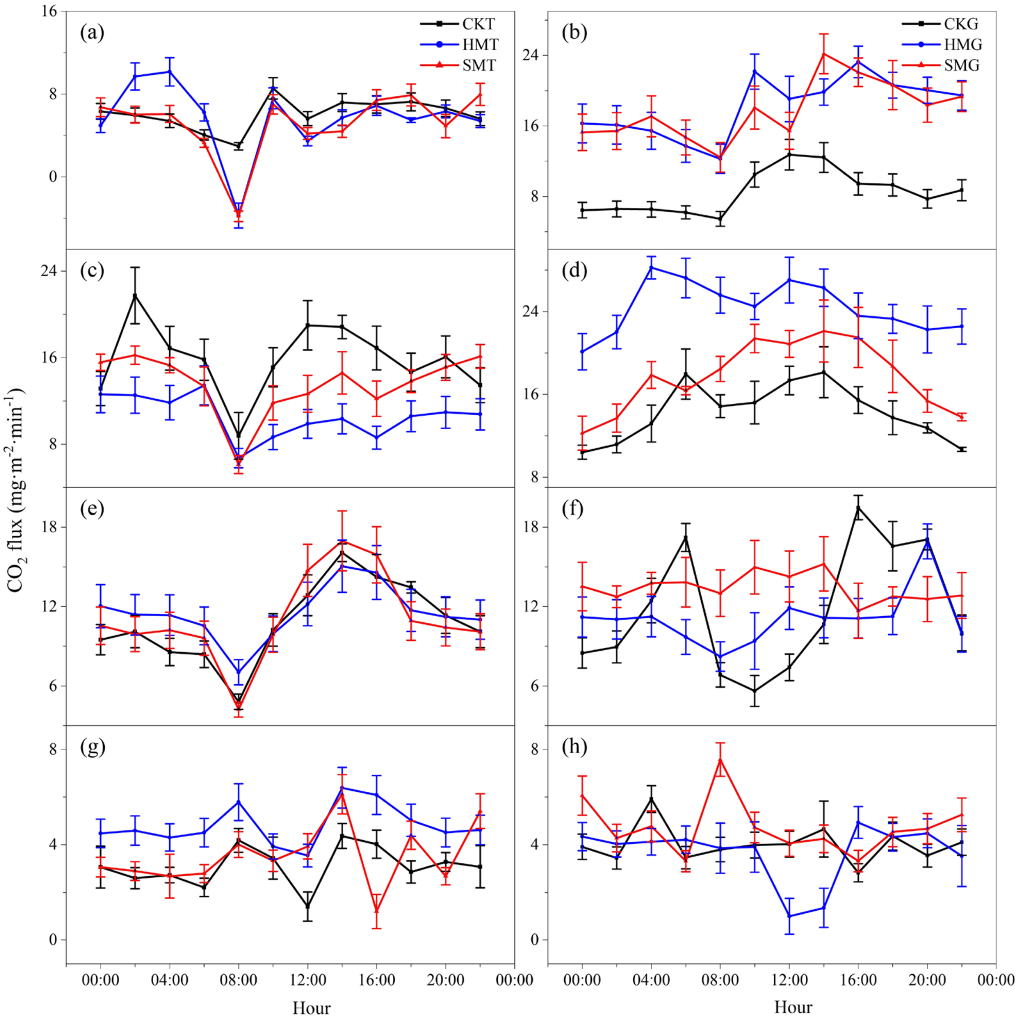


Figure 4. Diurnal variation in CO_2 fluxes from (a, c, e, g) tea rows and (b, d, f, h) inter-row zones under different green manure intercropping treatments across seasons: (a–b) spring, (c–d) summer, (e–f) autumn, and (g–h) winter. CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

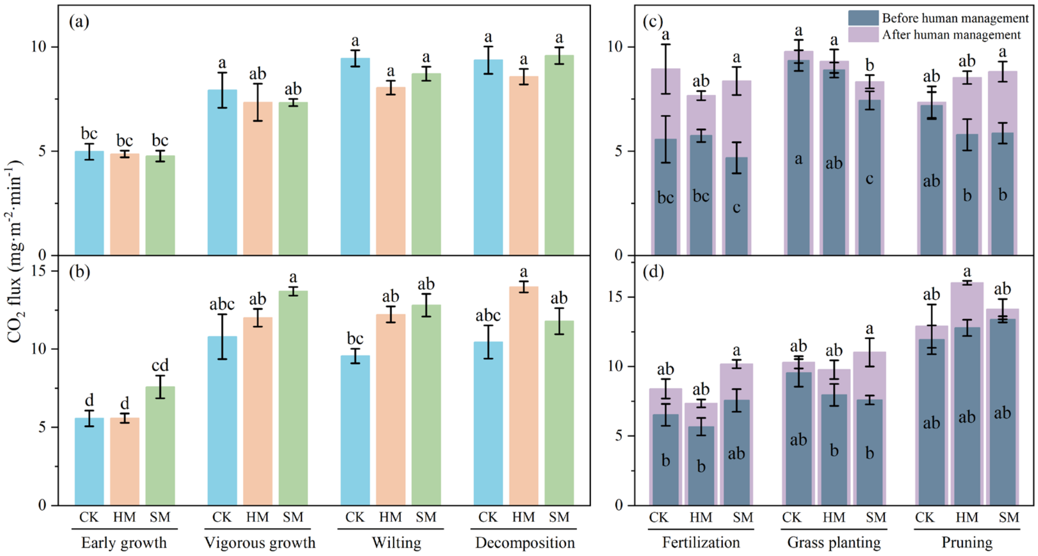


Figure 5. Temporal dynamics of CO_2 fluxes under green manure (a, b) growth stages and (c, d) management events in tea plantations. Growth stages include: early growth (mid-November to early April), vigorous growth (mid-April to late May), wilting (early June to late July), and decomposition (August). CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

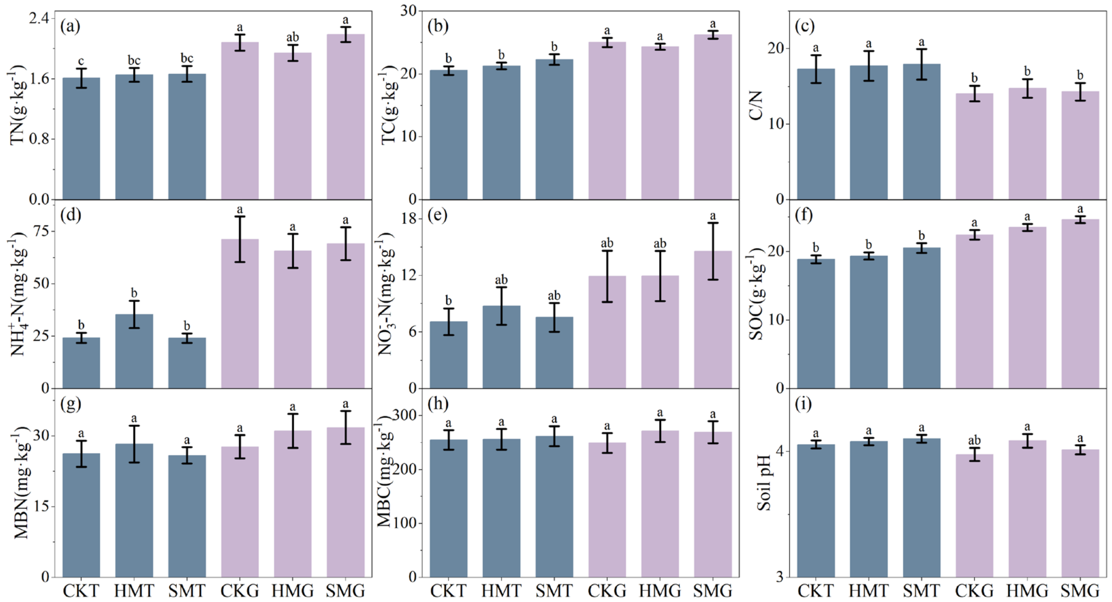


Figure 6. Basic physicochemical properties of soil in tea rows and inter-rows under different green manure intercropping treatments. CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

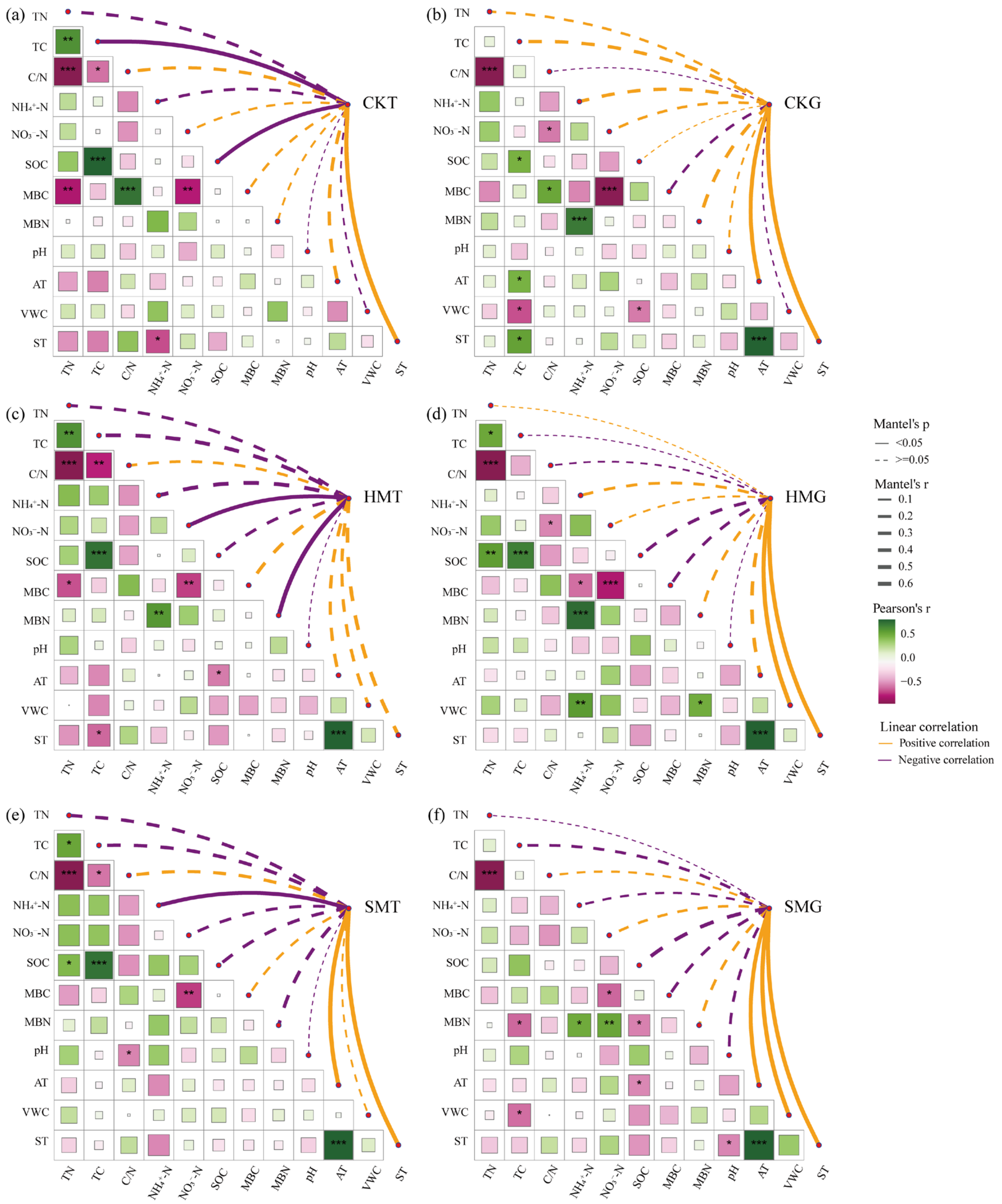


Figure 7. Pairwise correlations between environmental factors and their relationships with CO_2 fluxes under different green manure treatments (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

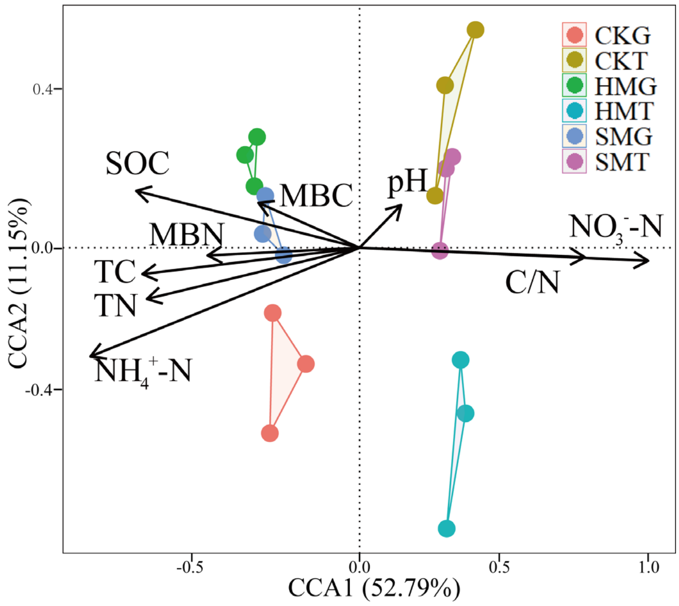


Figure 8. Canonical correspondence analysis (CCA) showing the influence of soil physicochemical properties on CO_2 emissions from tea rows and inter-rows under different green manure treatments. CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

Appendix 1

The CO_2 flux was calculated using the following equation:

$$F = \rho_0 \times \frac{P}{P_O} \times \frac{T_O}{T + T_O} \times \frac{V}{M} \times \frac{\Delta c}{\Delta t} \qquad (1)$$

where F is the CO_2 flux ($mg \cdot m^{-2} \cdot min^{-1}$); ρ_0 is the density of CO_2 under standard conditions ($1.98\ kg \cdot m^{-3}$); P_o and T_o are the standard atmospheric pressure (101.325 kPa) and temperature (273.15 K), respectively; P and T are the atmospheric pressure (kPa) and absolute temperature (K) at the time of sampling; V and M are the volume (m^3) and bottom area (m^2) of the chamber, respectively; $\Delta c/\Delta t$ is the slope of the linear or nonlinear regression of CO_2 concentration over time.

First, the raw CO_2 concentration readings were calibrated with standard gases. Then, linear regression was performed to fit their rate of change over time. Finally, CO_2 flux was calculated using the standard flux formula by incorporating the chamber volume, base area, and the measured atmospheric pressure and temperature.

~~Appendix 2~~

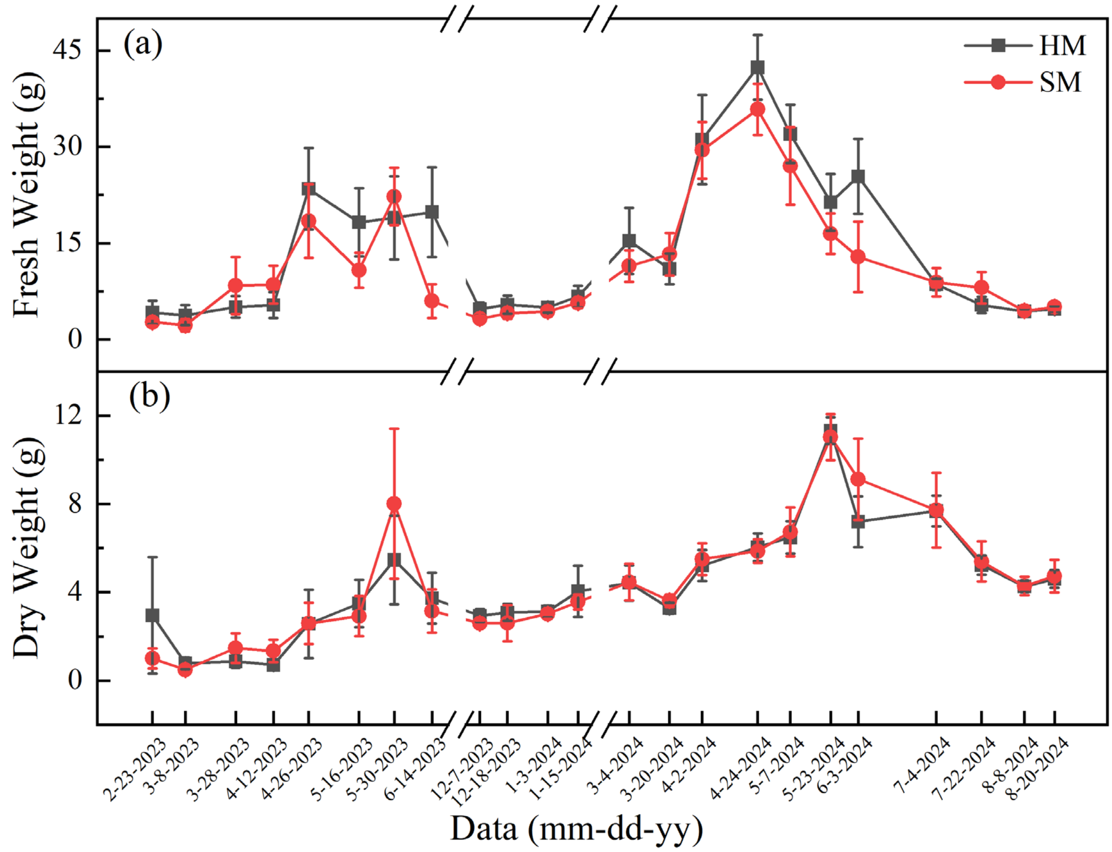


Figure A1. Temporal trend of green manure indexes

Spatially Contrasting CO_2 Dynamics Driven by Green Manure Intercropping in Subtropical Tea Plantations

Shuo Liu[1,4], Zeping Jin[1,3], Ziyi Chen[1,3], Haolin Li[1,3], Zihan Fan[3], Shaohui Li[3], Haiwang Fu[1,4], Wei He[1], Kunpeng Zang[1], Shuangxi Fang[1,5*], Peng Yan[2]

[1] Zhejiang Carbon Neutral Innovation Institute & Zhejiang International Cooperation Base for Science and Technology on Carbon Emission Reduction and Monitoring, Zhejiang University of Technology, Hangzhou 310014, China

[2] Key Laboratory of Tea Quality and Safety Control, Ministry of Agriculture, Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

[3] College of Environment, Zhejiang University of Technology, Hangzhou 310014, China

[4] Shaoxing Research Institute, Zhejiang University of Technology, Shaoxing 312077, China

[5] State Key Laboratory of Green Chemical Synthesis and Conversion, Zhejiang University of Technology, Hangzhou 310014, China

Correspondence authors:

Shuangxi Fang, E-mail: fangsx@zjut.edu.cn

Abstract:

Tea plantations are important contributors to greenhouse gas emissions due to intensive fertilization and continuous cultivation. However, the mechanisms by which green manure intercropping regulates soil CO_2 dynamics in these systems remain poorly understood. We employed the static chamber method over a two-year period, with sampling conducted weekly, to investigate how intercropping with *Vulpia myuros* (SM) and a legume–nonlegume mixture of *Lolium perenne* and *Trifolium repens* (HM) influenced spatial CO_2 flux dynamics compared with a no-intercropping control (CK) from tea rows and inter-row zones in a subtropical tea plantation. Distinct seasonal variations were observed, with CO_2 fluxes peaking in summer and autumn and declining in spring and winter. Average tea-row fluxes were 8.7% and 9.5% lower under SM and HM, respectively, compared to CK, indicating emission reductions with intercropping. In contrast, average inter-row fluxes increased by 19.4% under SM and 7.7% under HM, demonstrating pronounced spatial contrasts. Diurnal patterns generally exhibited midday peaks (12:00–14:00), especially in summer and autumn across all tea-rows, and short-term CO_2 pulses were triggered by field operations such as fertilization and pruning. Notably, HM effectively suppressed fertilization-induced CO_2 pulses, revealing the mitigation potential of legume–nonlegume mixtures. Green manure increased soil organic carbon (6.4%), lowered soil temperature (4.5%), and enhanced porosity (4.2%), collectively shaping CO_2 dynamics. Multivariate analysis identified soil organic carbon (SOC) and temperature as dominant flux drivers, and a potential SOC threshold was detected, beyond which CO_2 emissions accelerated. Compared to CK, intercropping reduced tea-row emissions by 7.1–7.9% but increased inter-row emissions by 12.7–28.9% based on the two years cumulative emissions, continuous intercropping significantly decreased overall inter-row emissions over time. These results highlight the spatially heterogeneous nature of carbon flux regulation and demonstrate the long-term potential of green manure intercropping as a climate-smart management strategy in perennial agroecosystems.

Keywords: tea plantations, green manure, CO_2 emissions, soil factors

1 Introduction

Mitigating greenhouse gas (GHG) emissions to address global warming and associated climate challenges remains a priority in global environmental research. Among long-lived GHGs, carbon dioxide (CO_2) plays the most prominent role, contributing approximately 66% to the increase in global radiative forcing (IPCC, 2022). In 2023, the global average atmospheric CO_2 concentration reached 420.0 ± 0.1 ppm, representing a 151% increase compared to pre-industrial levels (prior to 1750) (WMO, 2024). Agriculture is a major emission sector, accounting for about 14% of total anthropogenic CO_2 emissions (Wang et al., 2025). In China, this share is even higher, with agricultural activities accounting for up to 17% of national CO_2 emissions (Xu and Lin, 2017). Therefore, accurately characterizing CO_2 emission dynamics in agricultural systems and scientifically informed mitigation strategies are critical for advancing global GHG reduction efforts and promoting sustainable, low-carbon agricultural development (Xu et al., 2024).

Tea (*Camellia sinensis* L.) is an important economic crop in tropical and subtropical regions. Over recent decades, global tea cultivation area has expanded rapidly, reaching 4.70 million hectares in 2022. China has led the most significant growth, with 3.35 million hectares of tea plantations and an annual production of 2.82 million tons, ranking first worldwide in both area and output (FAO, 2024). To maximize yield and improve tea quality, fertilizer inputs to tea cultivation area can be up to four times higher than those applied to staple crops during a single growing season (Zou et al., 2009; Han et al., 2013; Yao et al., 2015). In China, average annual fertilizer use in tea plantations reaches 678 kg ha^{-1}, with more than 30% of plantations experiencing over-application (Ni et al., 2019). Such intensive fertilization not only accelerates soil acidification but also significantly increases GHGs emissions from tea plantations (Liu et al., 2016; Yan et al., 2020). However, most existing studies on agricultural CO_2 emissions have focused on staple cropping systems such as wheat (Song et al., 2024), rice (Qian et al., 2023), and maize (Zhang et al., 2020), while studies on CO_2 emissions from tea plantations remain limited.

For instance, Lang et al. (2017) reported that intercropping rubber trees with tea in the tropical forests of Xishuangbanna, China, reduced CO_2 emissions, although it simultaneously weakened the soil's methane (CH_4) uptake capacity. Wanyama et al. (2019) found that converting tropical montane forests into tea plantations in Africa decreased annual soil CO_2 emissions to 5.6 t ha^{-1}, with emissions positively correlated with soil pH and negatively correlated with the soil C/N ratio. Pang et al. (2019) quantitatively assessed the net ecosystem exchange (NEE) of tea plantations in southeastern China from 2014 to 2017, reporting values ranging from −182.40 to −301.51 g C m^{-2}, indicating that tea plantations act as net carbon sinks. However, their carbon sequestration potential was lower than that of other subtropical ecosystems, with temperature identified as the primary factor influencing ecosystem respiration. These findings suggest that CO_2 emissions from tea plantations play a non-negligible role on the carbon exchange between atmosphere and tea plantations. However, the limited number of studies has led to substantial uncertainty on estimating tea plantation CO_2 emissions, restricting our understanding of their contribution to regional and global agricultural GHG budgets (Li et al., 2016; Ji et al., 2020) and hindering the development of low-carbon tea plantations.

In response, there is a growing emphasis on the development of eco-friendly and low-carbon tea plantations (Wang et al., 2022). Toward reduction of fertilizer usage and higher economic efficiency, various management practices were incorporated, including by using green manure. As a modernized agricultural practice, green manure has been widely adopted in farming systems and serves as an important measure for improving soil quality, playing a vital role in sustainable agriculture. Within the context of GHGs mitigation, green manure is recognized as an effective solution for improving soil quality and enhancing CO_2 sequestration in agroecosystems (Forte et al., 2017). However, most studies examining the relationship between green manure and carbon emissions have focused on conventional croplands such as rice and wheat. Comprehensive studies have shown that appropriate green manure management can significantly reduce the global warming potential (GWP) associated with fertilization (Zhang et al., 2024). For instance, Gong et al. (2021) demonstrated that long-term ryegrass cover in organic soybean fields effectively reduced net GWP. In contrast, other studies have reported that green manure application may increase CO_2 emissions. Kim et al. (2013) found that the application of Chinese milk vetch and ryegrass increased winter CO_2 fluxes in paddy fields by approximately 197% and 266%, respectively. Large-scale assessments have further revealed that green manure tends to increase CO_2 emissions, primarily due to differences in plant species and biomass inputs. Biomass alone explained 63% of the variation in CO_2 emission increases, with emissions declining as the C/N ratio of cover crop biomass increased. Notably, mixed sowing of leguminous and non-leguminous green manures has been shown to improve residue C/N ratios and reduce GHGs emissions (Muhammad et al., 2019).

Existing studies on GHG mitigation in tea systems have predominantly focused on fertilizer reduction and substitution strategies. Wu et al. (2018) conducted a three-year fertilization control experiment in southern China and found that halving nitrogen input decreased nitrous oxide (N_2O) emissions by 44.5% in tea plantations. In contrast, Yao et al. (2015) reported that organic fertilizer application led to a 71% increase in N_2O emissions compared to conventional urea, suggesting potential trade-offs in GHG outcomes. Organic amendments, such as compost or manure, have been shown to improve soil fertility, enhance soil structure, porosity, and pH, and promote carbon sequestration in tea plantation soils (Han et al., 2013; Wu et al., 2021). Biochar application has also been identified as an effective strategy for improving soil quality while simultaneously enhancing soil carbon storage and reducing emissions (Wu et al., 2021). The effect of green manure intercropping on tea plantations was mainly focused on improvements in tea plant growth and soil nutrient dynamics. For example, intercropping with green manure species has been shown to enhance nitrogen use efficiency and increase soil microbial diversity (Huang et al., 2023). The potential role of green manure intercropping in mitigating GHGs emissions in tea ecosystems remains poorly understood, and its interactions with key environmental factors have not been fully clarified (Zhu et al., 2018). Green manure may influence CO_2 emissions by altering carbon input levels and inducing soil disturbances, but the specific emission characteristics and driving factors require further investigation.

To address these gaps, this study selected cultivated tea plantations region located in the east of China, where is recognized as a very important tea cultivation area famous by the tea name of Longjing. Commonly used green manure species in tea systems (*Vulpia myuros* C., *Lolium perenne* L., and *Trifolium repens* L.) were selected for intercropping, covering both leguminous and non-leguminous species, under monoculture and mixed-sowing configurations. CO_2 flux were carried out in both tea rows and inter-row zones across different intercropping treatments. Dynamics and key influencing factors of CO_2 emissions under various green manure intercropping models were analyzed, aiming to reveal the potential reasons by which green manure intercropping regulates carbon fluxes in tea plantations and provide support for the development of low-carbon tea plantations and sustainable regional agriculture.

2 Methodology

2.1 Monitoring Site

This study was conducted at the Comprehensive Experimental Tea Plantation Base of the Tea Research Institute, Chinese Academy of Agricultural Sciences, located in Shengzhou, Zhejiang Province, China (120°83′E, 29°75′N; elevation 30 m a.s.l.) (Fig. 1a). The site is situated in a low mountainous and hilly region of southeastern China and is characterized by a subtropical monsoon climate. During the experimental period (August 2022 to August 2024), the average annual temperature was approximately 16 °C, with an average annual precipitation of about 1400 mm. The region experiences a concentrated rainy season from April to June and has a frost-free period of around 240 days. The tea cultivated at the site is *Jinmudan*, an elite cultivar derived from the hybridization of *Tieguanyin* and *Huangdan*, and is widely planted across China. The tea plantation was established in 2015; the tea plants are 8–10 years old and arranged in a single-row planting pattern with a row spacing of 150 cm and a plant spacing of 40 cm. The soil type of the tea plantation is classified as red soil (Ultisol), a typical soil type in this region, and the initial basic physicochemical properties of the six treatment soils are shown in Table 1.

2.2 Experimental Setup

Three green manure intercropping treatments were established in this study: *Vulpia myuros* C. (SM), a mixture of *Lolium perenne* L. and *Trifolium repens* L. (HM), and a control treatment without intercropping (CK). The experimental tea plantation covered an area of approximately 1000 m^2. For each treatment, three representative tea rows were selected as three replicates. Adjacent treatments were separated by two tea rows (~3 m), and replicate areas within the same treatment were spaced approximately 5 m apart. Gas fluxes were measured in both tea rows (T) and inter-row zones (G), resulting in six treatments: SMT, SMG, HMT, HMG, CKT, and CKG, with a total of 18 representative sampling points (Fig. 1b, c). Tea plantation followed standard management practices, including fertilization, pruning, and tillage. Basal fertilization and tillage were conducted in middle of October, followed by green manure sowing in early November. Urea was applied as a topdressing in early February. Tea leaves were harvested in end of March, and pruning was normally conducted in May and July.

Gas sampling was performed using the static chamber–gas chromatography method. The dimensions of the static chambers were 1.25 × 0.8 × 1.0 m for tea rows and 0.3 × 0.3 × 0.5 m for inter-row zones (Fig. 1b). Each chamber was equipped with an internal fan to ensure uniform gas mixing. To avoid rapid heating due to sunlight, the chambers were wrapped with aluminum foil and sponge, functioning as dark chambers. To minimize disturbance, chamber bases with water grooves were installed one month in advance at each sampling point, inserted 15 cm into the soil. During sampling, water was added to the grooves, and the chamber was securely sealed onto the base to create a closed environment. Four gas samples were collected at 7-minute intervals using gas sampling bags.

For seasonal monitoring, sampling was conducted once per week between 09:00 and 11:00 a.m. (local time). Intensive sampling was also carried out following key management events such as fertilization and pruning. Diurnal variation was monitored over three consecutive days in January, April, July, and October, representing winter, spring, summer, and autumn, respectively. During these campaigns, gas samples were collected every 2 hours over a 24-hour period. All gas samples were analyzed within

24 hours by using a gas chromatograph (Agilent 7890B, Agilent Inc., USA). CO_2 concentrations were measured using a flame ionization detector (FID) at a working temperature of 175 °C. High-purity nitrogen was used as the carrier gas, with an injection volume of 30 mL and a flow rate of 250 mL·min^{-1}. During the tests, the deviation between the calculated regression values of CO_2 and the nominal mole fractions was 0.37 μmol mol^{-1}. The linear fit between the instrument response values and the nominal mole fractions achieved a correlation coefficient (R^2) of 0.9999. Furthermore, the standard gases used were calibrated in multiple rounds by the Greenhouse Gas Laboratory of the Atmospheric Observation Center of the China Meteorological Administration using primary standard gases, ensuring traceability to the World Meteorological Organization primary standards.

Meteorological data, including precipitation, atmospheric pressure, and air temperature (AT), were obtained from an automatic weather station installed within the tea plantation. Soil samples were recorded using an automatic weather station installed within the tea plantation. Soil samples were collected monthly using a five-point composite method within a 1 m radius of each sampling point. After passing through a 2 mm sieve, the samples were divided into three portions:

i. One fresh portion was analyzed for microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) using the chloroform fumigation-extraction method and a TOC analyzer.

ii. A second portion was air-dried and ground for analysis of soil pH.

iii. The third portion was stored at 4 °C for analysis of nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) by spectrophotometry, total carbon (TC) and total nitrogen (TN) by elemental analysis, and soil organic carbon (SOC) by the dichromate oxidation–spectrophotometry method.

Soil temperature (ST) and volumetric water content (VWC) were measured *in-situ* using a portable soil sensor (TDR-315H, Acclima). Soil bulk density (BD) and water-filled pore space (WFPS) were determined using the core ring method.

2.3 Data Processing

The flux refers to the amount of gas exchanged per unit time and unit area. A positive value indicates net emission to the atmosphere, while a negative value indicates net uptake from the atmosphere (Yao et al., 2015; Zhang et al., 2020). Based on the flux measurements, cumulative CO_2 emissions under different green manure intercropping treatments were also estimated. All data analyses and visualizations were performed using R software. Two-way analysis of variance (Two-way ANOVA) was employed to assess the effects of treatment type and observation period on CO_2 fluxes and soil physicochemical properties. Spearman correlation analysis and Mantel tests were used to examine the relationships between CO_2 flux and environmental variables under different green manure intercropping treatments. Canonical correspondence analysis (CCA) was applied to comprehensively evaluate the influence of soil physicochemical properties on CO_2 emissions. Data shown are means ± standard error (SE). In all statistical tests, the level of significant differences and correlations was set at $p < 0.05$.

3 Results

3.1 Long-term Variation of CO_2 Fluxes under Green Manure Intercropping

Figure 2 illustrates the long-term trends of key environmental variables and CO_2 fluxes in the tea plantation throughout the observation period. Overall, CO_2 fluxes from both tea-row and inter-row zones displayed distinct seasonal patterns: higher in summer and autumn, and lower in spring and winter. The seasonal differences between the warm (summer and autumn) and cool (spring and winter) periods were statistically significant (Table 2). The temporal dynamics of CO_2 fluxes closely tracked the trends in air temperature (Fig. 2a), suggesting that temperature is a key driver of soil respiration in tea plantations. Annual fluctuations in CO_2 fluxes were also strongly influenced by field management activities. For example, a sharp increase in CO_2 emissions was observed following basal fertilization in October, and another rise occurred in March of the following year after topdressing and with the onset of warmer temperatures, ultimately peaking in summer (Fig. 2b-c). The effects of management activities are further detailed in Section 3.3.

In tea rows, the annual mean CO_2 fluxes under HMT and SMT treatments were 7.35 ± 0.44 mg·m^{-2}·min^{-1} and 7.41 ± 0.45 mg·m^{-2}·min^{-1}, respectively, both lower than that of the control (CKT: 8.12 ± 0.46 mg·m^{-2}·min^{-1}) (Fig. 2b). In contrast, in inter-row zones, the annual mean CO_2 fluxes were significantly higher under HMG (9.77 ± 0.54 mg·m^{-2}·min^{-1}) and SMG (10.83 ± 0.52 mg·m^{-2}·min^{-1}) compared to the control (CKG: 9.07 ± 0.44 mg·m^{-2}·min^{-1}) ($p < 0.05$) (Fig. 2c). Across seasons, CKT generally exhibited higher CO_2 fluxes than HMT and SMT, except during winter. In the inter-row zones, both HMG and SMG showed significantly higher fluxes than CKG in summer, while SMG consistently had significantly higher CO_2 emissions than both CKG and HMG during the remaining seasons ($p < 0.05$) (Table 2).

Overall, green manure intercropping significantly increased CO_2 emissions from inter-rows, but reduced emissions in tea rows. In terms of cumulative annual emissions, HMT and SMT resulted in 3.69 kg·m^{-2} and 3.66 kg·m^{-2} of CO_2 emissions, respectively, both lower than the 3.97 kg·m^{-2} under CKT (Fig. 3). Similarly, cumulative CO_2 emissions under HMG and SMG remained consistently higher than under CKG, but they declined from 5.76 kg·m^{-2} and 6.43 kg·m^{-2} in the first year to 4.16 kg·m^{-2} and 4.92 kg·m^{-2} in the second year, respectively (Fig. 3). Two consecutive years of green manure intercropping led to a gradual reduction in CO_2 emissions from inter-rows, indicating its potential role in long-term emission mitigation in tea plantations. CO_2 emissions from inter-rows were substantially higher than those from tea rows. Compared with the control, HM and SM intercropping increased inter-row cumulative CO_2 emissions by 12.7% and 28.9%, respectively, while reducing tea-row emissions by 7.1% and 7.9% (Fig. 3a-b). Inter-row zones accounted for 52.6%, 57.3%, and 60.8% of the total annual CO_2 emissions in the CK, HM, and SM treatments, respectively (Fig. 3c-d), indicating that the inter-row emissions cannot be ignored.

3.2 Diurnal CO_2 Variations

CO_2 fluxes in the tea plantation exhibited pronounced diurnal variations across all seasons, particularly in spring and summer (Fig. 4), likely influenced by the growth stages of green manure species. In spring, CO_2 fluxes in tea rows under all treatments showed a similar diurnal trend: an initial decline followed by a rapid increase. HMT and SMT reached their minimum fluxes at 08:00 (local time), with values of –3.74 $mg\cdot m^{-2}\cdot min^{-1}$ and –3.80 $mg\cdot m^{-2}\cdot min^{-1}$, respectively, then rose sharply and stabilized in the afternoon. The diurnal amplitudes under HMT and SMT were notably greater than that of the control (CKT) (Fig. 4a). In the inter-row zones, the diurnal patterns under green manure treatments differed notably from the control (Fig. 4b). CKG displayed a unimodal pattern with a peak at 12:00 (12.74 $mg\cdot m^{-2}\cdot min^{-1}$) and a trough at 08:00 (5.45 $mg\cdot m^{-2}\cdot min^{-1}$), resulting in an amplitude of 7.29 $mg\cdot m^{-2}\cdot min^{-1}$. In contrast, HMG and SMG exhibited later peaks at 16:00 (23.26 $mg\cdot m^{-2}\cdot min^{-1}$) and 14:00 (24.17 $mg\cdot m^{-2}\cdot min^{-1}$), respectively, with troughs also at 08:00 (HMG: 12.28 $mg\cdot m^{-2}\cdot min^{-1}$; SMG: 12.43 $mg\cdot m^{-2}\cdot min^{-1}$). Both treatments showed substantially higher amplitudes than CKG.

Summer exhibited the most pronounced diurnal variation of CO_2 fluxes across all seasons. In tea rows, CKT, HMT, and SMT followed a bimodal pattern, with peaks at 02:00 and 12:00, and a trough at 08:00. Their respective diurnal amplitudes were 12.96, 6.70, and 10.10 $mg\cdot m^{-2}\cdot min^{-1}$ (Fig. 4c). In the inter-rows, the amplitudes were relatively lower, 7.72, 8.12, and 7.79 $mg\cdot m^{-2}\cdot min^{-1}$ for CKG, HMG, and SMG, respectively, indicating smaller fluctuations compared to tea rows (Fig. 4d). Notably, summer also showed the most distinct contrast between tea rows and inter-rows: CKT recorded the highest average flux in the tea rows, while CKG had the lowest in the inter-rows.

In autumn, tea-row fluxes under all treatments exhibited a unimodal pattern, with minima at 08:00 and peaks at 14:00. The diurnal amplitudes were 11.27, 8.02, and 12.75 $mg\cdot m^{-2}\cdot min^{-1}$ for CKT, HMT, and SMT, respectively (Fig. 4e). In the inter-rows, HMG and SMG displayed relatively stable diurnal trends, whereas CKG showed a bimodal pattern with peaks at 06:00 and 16:00, and a greater amplitude than both HMG and SMG (Fig. 4f).

In winter, CO_2 fluxes showed the most stable diurnal variation of the year. In tea rows, amplitudes were only 2.96, 2.84, and 4.92 $mg\cdot m^{-2}\cdot min^{-1}$ for CKT, HMT, and SMT, respectively (Fig. 4g). Unlike other seasons, 08:00 no longer corresponded to the daily minimum but rather to a relative maximum, with daily peaks generally occurring at 14:00. In inter-rows, diurnal patterns were less defined. SMG exhibited the highest flux at 08:00 (7.57 $mg \cdot m^{-2} \cdot min^{-1}$), while HMG showed the lowest at 14:00 (0.99 $mg \cdot m^{-2} \cdot min^{-1}$) (Fig. 4h).

3.3 Effect of Human Management on CO_2 Fluxes

CO_2 fluxes from the tea field varied significantly across different growth stages of green manure, exhibiting a general increasing trend from the early growth stage to the vigorous, wilting, and decomposition stages (Fig. 5a). In the tea rows, the lowest fluxes were observed during the early growth stage, while the highest occurred during the decomposition stage. Differences among the three treatments (CKT, HMT, and SMT) were minimal during the early growth but became more apparent in the subsequent stages. Notably, during the vigorous stage, both HMT and SMT treatments reduced CO_2 emissions compared to CKT. In contrast, the impact of green manure growth on CO_2 fluxes was more pronounced in the inter-row zones (Fig. 5b). At all growth stages, CO_2 fluxes under the HMG and SMG treatments were significantly higher than those under CKG, with the largest differences observed during the wilting stage. Peak emissions occurred during the decomposition stage for HMG and during the wilting stage for SMG.

Fertilization substantially increased CO_2 emissions across the tea plantation (Fig. 5c-d). In tea rows, the post-fertilization increase in CO_2 flux under HMT was 43.1% lower than that under CKT, whereas SMT showed a 9.2% higher increase. In the inter-row zones, HMG reduced the fertilization-induced increase by 10.4% compared to CKG, while SMG amplified it by 40.1%. These findings indicate that the HM treatment can effectively mitigated CO_2 emissions triggered by fertilization, while SM treatment may intensify them, revealing the potential of legume-based mixed green manure to reduce CO_2 emissions in tea plantations. It is worth noting that the mitigation effect in the inter-row zones was weaker than in the tea rows, possibly due to differences in root distribution or organic matter inputs.

The effects of grass planting and tea pruning on CO_2 fluxes varied by treatment type and location (tea row or inter-row) (Fig. 5c-d). In the CK treatment, grass planting had no significant impact on CO_2 fluxes. However, the HM treatment led to a marked increase after grass planting, with inter-row fluxes rising by 1.81 $mg \cdot m^{-2} \cdot min^{-1}$. Similarly, the SM treatment showed significant increases in both zones, with an increase of 0.90 $mg \cdot m^{-2} \cdot min^{-1}$ in tea rows and an inter-row increase that was 3.8 times greater. These increases can be attribute to soil disturbance during sowing.

After tea pruning, no significant changes in CO_2 flux were observed in the CK treatment. However, both HMT and SMT significantly increased CO_2 emissions in tea row, with increments of 2.74 $mg \cdot m^{-2} \cdot min^{-1}$ and 2.94 $mg \cdot m^{-2} \cdot min^{-1}$, respectively. In the inter-row zones, only the HMG treatment exhibited a significant post-pruning increase of 3.25 $mg \cdot m^{-2} \cdot min^{-1}$. These increases may be attributed to pruning residues covering the green manure surface, which could elevate soil temperature and moisture, thereby enhancing soil respiration and CO_2 emissions.

3.4 Effects of Environmental Factors on CO_2 Fluxes

Significant differences in soil nutrient parameters were observed between tea rows and inter-row zones under various green manure intercropping treatments (Fig. 6). Green manure treatments generally increased soil total carbon (TC) and total nitrogen (TN), with consistently higher TC and TN levels in the inter-row zones than in the tea rows (Fig. 6a-b), resulting in significantly higher C/N ratios in the tea rows (Fig. 6c). Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) concentrations were also significantly greater in the inter-row zones, with the highest NH_4^+-N found in CKG (71.20 $mg \cdot kg^{-1}$) and the highest NO_3^--N in SMG (14.56 $mg \cdot kg^{-1}$). All green manure treatments significantly increased soil organic carbon (SOC), the average SOC contents under HM and SM were 3.6% and 9.3% higher than under CK, respectively (Fig. 6f). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) showed no significant differences between tea rows and inter-row zones, but both were slightly elevated under green manure treatments (Fig. 6g-h). Soil pH ranged from 3.6 to 4.5, with no significant differences among treatments, although green manure application slightly increased soil pH (Fig. 6i).

During the monitoring, soil temperature ranged from 2.3–41.8°C in CK, 3.0–37.6°C in HM, and 3.5–36.6°C in SM. Average soil temperatures in HM and SM were 4.5% and 3.9% lower than in CK, indicating a cooling effect of green manure. Additionally, bulk density was reduced by 8.9% and 5.0% in HM and SM compared to CK, while total porosity increased by 5.3% and 3.0%, and WFPS decreased by 29.1% and 11.1%, respectively. These results suggest that green manure intercropping effectively reduces soil compaction and improves soil aeration. The combined effect of these factors is the key to the changes in CO_2 emissions.

To further clarify these relationships, we examined the correlations between CO_2 fluxes and environmental factors under different green manure treatments (Fig. 7). CO_2 fluxes across nearly all treatments were significantly positively correlated with air temperature (AT) and soil temperature (ST) ($r > 0.5$, $p < 0.05$), suggesting temperature as a major driver of CO_2 emissions in tea plantations. Treatment-specific differences were also apparent: in green manure treatments (HM and SM), both TC and SOC in tea rows and inter-row zones showed negative correlations with CO_2 flux, whereas volumetric water content (VWC) showed significant positive correlation ($r > 0.5$, $p < 0.05$). In contrast, VWC was negatively correlated with CO_2 flux under CK treatment. These findings suggest that green manure intercropping alters soil pore structure and moisture regimes, thereby modifying CO_2 emission dynamics compared to bare soil conditions. Additionally, environmental controls on CO_2 fluxes differed between tea rows and inter-row zones. Emissions in tea rows appeared less sensitive to environmental fluctuations, likely due to the moderating effects of tea canopy coverage and root systems.

Canonical correspondence analysis (CCA) was further performed to examine the effect of green manure intercropping patterns and soil properties on CO_2 emissions in tea rows and inter-row zones (Fig. 8). The first two CCA axes explained 52.79% and 11.15% of the total variance, respectively. CCA1 was primarily associated with NH_4^+-N, NO_3^--N, SOC, and the C/N ratio, while CCA2 was mainly linked to TN, TC, MBN, MBC, and pH. These results indicate distinct environmental drivers of CO_2 emissions between the two spatial zones. In tea rows, CO_2 flux was positively associated with

NO_3^--N and the C/N ratio, with relatively minor influence from pH. The influencing soil factors were similar for CKT and SMT, whereas HMT displayed a distinct pattern, likely attributable to the presence of leguminous green manure. In inter-row zones, SOC emerged as the dominant factor controlling CO_2 fluxes in HMG and SMG treatments, whereas NH_4^+-N was the key driver in CKG. Moreover, TC, TN, MBC, and MBN all showed positive associations with inter-row CO_2 fluxes, with consistent soil drivers under HMG and SMG that differed from CKG, indicating that green manure significantly affects soil–CO_2 interactions.

4 Discussion

4.1 CO_2 Flux Dynamics under Green Manure Intercropping

This study revealed pronounced seasonal variations in CO_2 fluxes from tea plantations, which were closely aligned with fluctuations in air temperature (Fig. 2a-c). In spring, rising temperatures enhanced both plant and microbial respiration, leading to a sharp increase in CO_2 emissions (Yan et al., 2022). During summer, when temperatures reached their annual peak, intensified microbial activity accelerated the decomposition of soil organic matter, resulting in the highest CO_2 fluxes of the year (Allison et al., 2010). In autumn, declining temperatures and light availability reduced microbial activity and soil respiration, thereby lowering CO_2 emissions (Liu et al., 2020). In winter, low temperatures significantly inhibited both plant and microbial respiration, causing CO_2 fluxes to drop to their annual minimum (Schnecker et al., 2023). These seasonal flux patterns were consistent throughout the two-year observation period, indicating the pivotal role of temperature in regulating CO_2 emissions in tea plantations (Chen et al., 2021).

Compared with the CK treatment, HM and SM increased annual cumulative CO_2 emissions by 3.3% and 7.9%, respectively, revealing that green manure intercropping significantly elevated total CO_2 emissions. Similar findings have been reported in previous studies. For example, Lee et al. (2021) observed consistently higher cumulative CO_2 emissions in cropland soils under green manure treatments than under fallow conditions. A meta-analysis by Muhammad et al. (2019) also showed that the use of cover crops generally increases CO_2 emissions compared with bare soil. This effect can be attributed to two possible mechanisms: 1) green manure crops introduce exogenous carbon inputs into the soil, which stimulates CO_2 release (Ho et al., 2021); and 2) green manure intercropping reduces soil bulk density and increases total porosity (Song et al., 2016), thereby improving soil aeration and promoting aerobic microbial activity. The enhanced microbial activity accelerates the decomposition and mineralization of soil organic matter, consequently increasing CO_2 emissions (Chen et al., 2019). By contrast, under the CK treatment, higher WFPS and greater soil compaction may have inhibited gas diffusion and limited CO_2 release into the atmosphere (Lang et al., 2017).

Diurnal variations of CO_2 fluxes were influenced by both seasonal dynamics and the growth stages of green manure crops. CO_2 fluxes fluctuated most sharply during spring and summer, with temperature identified as the primary driver of daily flux patterns (Pang et al., 2019). In spring, negative CO_2 flux peaks were observed in tea rows under HMT and SMT around 08:00, due to low morning temperatures suppressing microbial respiration. Moreover, the presence of easily degradable organic matter from green manure may have diverted microbial metabolism toward biomass accumulation rather than complete mineralization to CO_2. In summer, distinct spatial differences appeared between tea rows and inter-row zones. In tea rows, CO_2 fluxes under the CKT treatment were significantly higher than those under HMT and SMT, while in the inter-row zones, fluxes under HMG and SMG were higher than those under CKG. This spatial heterogeneity highlights the dual role of green manure: in tea rows, the shading effect of green manure canopy reduced soil temperatures, thereby inhibiting microbial respiration; in contrast, inter-row zones were exposed to direct sunlight, root exudates and decomposing plant residues provided additional carbon sources. Under favorable thermal conditions, this stimulated microbial activity and thus increased CO_2 emissions (Gui et al., 2024). In autumn and winter, CO_2 flux peaks were mostly recorded in the afternoon, possibly due to rising temperatures reaching a threshold that accelerated enzymatic reactions and microbial metabolism, enhancing root and soil respiration and thus elevating CO_2 emissions (Dove et al., 2021).

4.2 Influence of Cultivation Management on CO_2 Fluxes

Fertilization, pruning, and soil tillage with cover cropping are critical anthropogenic management practices in tea plantations that significantly affect CO_2 flux dynamics. Fertilizer application, in particular, is a major contributor to agricultural greenhouse gas emissions, with emission strength influenced by the type, amount, and method of application (Wang et al., 2024). In this study, the application of rapeseed cake and compound fertilizers significantly increased soil CO_2 fluxes, especially within tea rows. This increase can be attributed to two main factors: 1) the input of exogenous organic matter enriched soil organic carbon content; and 2) trench fertilization caused physical disturbance, disrupting soil aggregates and accelerating the decomposition of soil organic carbon. These disturbances stimulated the abundance and metabolic activity of aerobic heterotrophic microbes, promoting organic matter mineralization and resulting in elevated CO_2 emissions (Chappell et al., 2015; Struck et al., 2020). Intensive pruning conducted in May and August further contributed to increased CO_2 fluxes. Pruning substantially reduces the photosynthetic biomass of tea plants, diminishing their carbon sequestration capacity. Simultaneously, the resulting litterfall provides abundant substrates for microbial respiration (Pang et al., 2019). The combined effect of reduced carbon uptake and increased decomposition substrates leads to a rapid short-term increase in CO_2 emissions after pruning.

Previous studies have demonstrated that intercropping systems introduce readily decomposable carbon through root exudates, while green manure decomposition increases organic matter inputs and improves soil organic carbon storage (Gui et al., 2024). In this study, CO_2 fluxes in inter-row zones were significantly higher than the control during the wilting and decomposition stages of green manure, suggesting that microbial activity was enhanced during these periods, thereby accelerating the decomposition and transformation of organic matter and intensifying soil respiration. Additionally, soils under green manure treatments exhibited lower annual average temperatures compared to the control, indicating that intercropping with green manure moderated surface soil temperatures and reduced daily temperature fluctuations. This effect was particularly pronounced in summer, when green manure not only reduced inter-row CO_2 emissions but also improved the microclimatic conditions of the tea plantation.

Traditional CO_2 flux measurements in tea plantations have mostly focused only on tea rows, often neglecting inter-row soil emissions (Yao et al., 2015; Chen et al., 2021). In this study, static chambers were parallelly employed to measure CO_2 fluxes in tea rows and inter-row areas, enabling a more accurate understanding of CO_2 emissions. Results showed that inter-row CO_2 fluxes were significantly higher than those in tea rows ($p < 0.05$), accounting for 52.6%, 57.3%, and 60.8% of the annual cumulative CO_2 emissions under CK, HM, and SM treatments, respectively. These findings emphasize the substantial contribution of inter-row zones to overall CO_2 emissions. This discrepancy is due to differences in management intensity: fertilization and tillage are commonly performed in inter-row areas, while the soil beneath tea canopies experiences minimal disturbance (Hirono and Nonaka, 2012). Moreover, pruning residues often accumulate in inter-row zones, further intensifying microbial activity and CO_2 emissions in these areas. The cumulative CO_2 emissions under HMG and SMG treatments were significantly lower in the second year. The decrease in emissions can be attributed to the gradual attenuation of the carbon priming effect induced by soil disturbance during the initial experimental phase (Zhou, 2025), coupled with the long-term positive effects of green manure on enhancing soil physical structure and ecosystem stability (Gui et al., 2024). The increase in green manure biomass in the following year indicates that the green manure system is transitioning from an initially disturbed and unstable state toward a more productive and carbon-sequestration-enhanced stable state (Figure A1). This trend not only reflects the improved functioning of the soil ecosystem but also serves as an important driver for further carbon sequestration, contributing significantly to the reduction in inter-row CO_2 emissions observed in the following year. Therefore, long-term and systematic monitoring of inter-row soil CO_2 emissions is essential for accurately assessing the carbon dynamics and mitigation potential of tea plantation ecosystems.

4.3 Differences in Environmental Drivers

Soil CO_2 fluxes are regulated by multiple environmental factors, including photosynthetic activity or vegetation productivity (Tang et al., 2005), and soil properties such as temperature and moisture (Liu et al., 2023; Widanagamage et al., 2025). Among them, temperature is widely recognized as a primary driver of seasonal variation in soil respiration (Lang et al., 2017). Our results showed that CO_2 fluxes in both tea rows and inter-row areas were significantly correlated with soil and air temperatures under different green manure treatments (Fig. 7). In addition, carbon and nitrogen transformation processes driven by microorganisms are closely coupled. Nitrification and denitrification alter NO_3^-–N and NH_4^+–N levels, thereby influencing soil physicochemical properties and microbial activity. As a result, CO_2 emissions exhibit significant positive correlations with nitrogen mineralization, denitrification, and N_2O emissions (Dai et al., 2020). This carbon–nitrogen coupling may interact with the distinctive nutrient uptake characteristics of tea plants, which are ammonium-preferring species with rapid NH_4^+ assimilation (Xin et al., 2024). In our study, CO_2 fluxes under the CKG treatment were positively correlated with NH_4^+–N content (Fig. 9). NH_4^+–N levels under this treatment (71.20 mg·kg^{-1}) were significantly higher than in the SMG and HMG treatments, whereas the corresponding soil pH value (3.97) was significantly lower ($p < 0.05$). This concurrent high NH_4^+–N level and strong acidification is due to ammonium accumulation under conventional fertilization and subsequent H^+ release during nitrification (Chen et al., 2021). By contrast, the soil pH under green manure intercropping treatments increased by 0.02–0.11 units compared to the CK treatment (Fig. 6i), suggesting that root exudates and organic matter inputs from green manure buffered soil acidification by reducing H^+ release during NH_4^+ nitrification. Moreover, the NO_3^-–N concentration in the SMG treatment (14.56 mg·kg^{-1}) was significantly higher than that in other treatments (Fig. 6e), due to the high biomass of *Vulpia myuros* C., which may reduce nitrate losses via runoff or leaching. Its active root system also improved soil aeration, inhibiting denitrification under anoxic conditions.

SOC content reflects the dynamic balance between organic matter inputs and decomposition (Mo et al., 2024). In our study, SOC levels in the HMT and SMT treatments were higher than those in the CKT treatment, while their cumulative annual CO_2 emissions were lower. This indicates that increasing SOC storage can help mitigate greenhouse gas emissions, consistent with findings by Han et al. (2022). However, the HMG and SMG treatments exhibited much higher SOC levels than CKG, while their cumulative CO_2 emissions exceeded those of CKG. This implies that once SOC accumulation surpasses a certain threshold, the excess carbon supply may stimulate microbial activity and subsequently increase CO_2 emissions (Lim and Choi, 2014). Interestingly, recent studies reveal that SOC thresholds can modulate the impact of nitrogen fertilization on carbon sequestration. In SOC-poor soils, nitrogen inputs tend to promote carbon accumulation and soil aggregation, enhancing SOC storage. Conversely, in SOC-rich soils, nitrogen fertilization may enhance microbial metabolic efficiency and increase microbial residue production (Ling et al., 2025). Studies in different climatic zones of China have revealed that SOC thresholds are influenced by factors such as climate and soil type. In the maritime monsoon climate zone, dual thresholds for NO_3^--N and extractable iron (Fe) have been identified, beyond which their marginal effects on SOC shift significantly. In the continental monsoon climate zone, SOC content increases markedly once a critical threshold of TN is exceeded (Cui, 2025). Additionally, research in alpine ecosystems has shown that SOC components vary along elevation gradients and exhibit distinct thresholds (Zhang, 2025). These insights provide a new perspective for interpreting our results and highlight the importance of identifying threshold values under multifactorial interactions to better assess their effects on CO_2 emissions.

In tea rows, excessively high soil C/N ratios may result in nitrogen limitation, thereby inhibiting rapid decomposition of organic matter and reducing CO_2 fluxes. Green manure, as a fresh plant residue with a relatively low C/N ratio, can be rapidly decomposed by soil microbes after incorporation, thus maintaining or enhancing SOC levels (Li et al., 2024), which aligns with our observations (Fig. 7d). MBC and MBN are generally considered closely linked to SOC (Gao et al., 2022). However, in our study, a significant positive correlation between MBC and SOC was only observed in the CKT treatment. The lack of correlation under green manure treatments may be due to the rapid and excessive input of exogenous carbon, which complicates the relationship between these variables. No significant differences in MBC and MBN levels were found among green manure treatments, and both showed weak correlations with CO_2 fluxes (Fig. 8), suggesting that MBC and MBN are not key drivers of CO_2 emissions in tea plantations.

5 Conclusion

This study revealed the regulation of CO_2 fluxes in tea plantations under different green manure intercropping treatments. Green manure significantly influenced CO_2 flux dynamics, with pronounced seasonal variations, higher fluxes in summer and autumn and lower fluxes in spring and winter. CO_2 emissions from inter-row areas were consistently higher than those from tea rows. CO_2 fluxes in the SM and HM treatments were significantly lower than in the CK treatment within tea rows, while the opposite trend was observed in inter-row areas, suggesting distinct spatial responses to green manure intercropping. Over the observation period, the HM and SM treatments reduced CO_2 emissions from tea rows by 7.1%–7.9% compared to the CK, while increasing inter-row emissions by 12.7%–28.9%. Inter-row CO_2 emissions accounted for 52.6%, 57.3%, and 60.8% of the annual cumulative CO_2 fluxes under the CK, HM, and SM treatments, respectively. These findings highlight the importance of incorporating spatial emission weighting into carbon accounting for agricultural ecosystems.

The observed spatial differences in CO_2 fluxes were closely related to variations in SOC content. Our findings suggest the existence of a critical SOC threshold that determines whether CO_2 emissions increase or decrease. Future research should focus on quantifying such thresholds under multi-factor interactions to better assess their impacts on greenhouse gas dynamics. Continuous green manure intercropping over two years significantly reduced inter-row CO_2 emissions, and the HM treatment suppressed fertilization-induced emission peaks. These findings demonstrate that green manure intercropping, particularly mixed legume and non-legume combinations, can effectively alter the spatial pattern of CO_2 emissions and mitigate carbon losses. Moreover, this practice provides significant co-benefits like improved soil aeration and fertility, reduced chemical fertilizers, weed suppression, and promoted tea plant growth, thereby offsetting the extra costs in labor and seeds. Therefore, green manure intercropping emerges as a practical and multifunctional strategy for reducing carbon emissions in agroecosystems.

Data availability

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

Author contributions

S. Liu conceived and designed the study, performed the data analysis, and drafted the manuscript. S. Liu, Z. Jin, and Z. Chen contributed to data visualization. H. Li, Z. Fan, S. Li, H. Fu, K. Zang, W. He, and P. Yan conducted field and laboratory work. S. Fang supervised the research, provided funding, and contributed to manuscript review and editing.

Competing interests

The contact author has declared that none of the authors has any competing interests.

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Tables and Figures

Table 1. Initial basic physicochemical properties of the six treatments.

Tpye	pH	NH_4^+–N ($mg\cdot kg^{-1}$)	NO_3^-–N ($mg\cdot kg^{-1}$)	TN ($g\cdot kg^{-1}$)	TC ($g\cdot kg^{-1}$)	SOC ($g\cdot kg^{-1}$)
CKT	4.25 ± 0.06^{a}	29.67 ± 7.50^{b}	5.01 ± 2.02^{b}	2.16 ± 0.16^{ab}	22.93 ± 2.00^{a}	25.50 ± 2.65^{a}
HMT	4.01 ± 0.06^{ab}	37.33 ± 4.43^{ab}	6.32 ± 2.25^{b}	2.01 ± 0.09^{ab}	21.08 ± 1.12^{a}	22.00 ± 1.69^{a}
SMT	4.11 ± 0.06^{abc}	30.42±8.93b	5.10 ± 1.88^{b}	1.96 ± 0.12^{b}	20.47 ± 1.12^{a}	21.83 ± 1.92^{a}
CKG	4.16 ± 0.02^{ab}	33.20 ± 5.90^{ab}	8.59 ± 1.46^{ab}	2.41 ± 0.19^{ab}	25.70 ± 1.99^{a}	21.68 ± 1.82^{a}
HMG	3.98 ± 0.05^{b}	37.90 ± 7.98^{ab}	12.73 ± 3.94^{a}	2.70 ± 0.35^{a}	27.77 ± 3.57^{a}	27.98 ± 3.55^{a}
SMG	4.00 ± 0.03^{b}	44.40 ± 7.33^{a}	13.36 ± 3.82^{a}	2.31 ± 0.28^{ab}	24.92 ± 2.86^{a}	26.88 ± 2.53^{a}

*Data shown are means ± SE. Different superscript letters indicate the significant difference ($p < 0.05$). CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

Table 2. Seasonal variation in CO_2 fluxes from tea rows and inter-row zones under different green manure intercropping treatments.

Type	Spring (mg·m^{-2}·min^{-1})	Summer (mg·m^{-2}·min^{-1})	Autumn (mg·m^{-2}·min^{-1})	Winter (mg·m^{-2}·min^{-1})
CKT	6.46 ± 0.58^{bcd}	9.66 ± 0.49^{a}	9.93 ± 0.68^{a}	3.60 ± 0.36^{f}
HMT	5.65 ± 0.44^{def}	7.97 ± 0.39^{abc}	9.21 ± 0.46^{a}	4.10 ± 0.43^{ef}
SMT	6.05 ± 0.39^{cde}	8.99 ± 0.34^{a}	8.47 ± 0.30^{ab}	3.71 ± 0.37^{f}
CKG	8.83 ± 1.14^{cd}	10.63 ± 0.66^{abc}	9.76 ± 0.83^{ab}	4.03 ± 0.52^{e}
HMG	9.08 ± 0.49^{cd}	13.03 ± 0.29^{a}	9.38 ± 0.39^{bcd}	4.19 ± 0.29^{e}
SMG	10.76 ± 0.43^{ab}	12.69 ± 0.73^{ab}	10.94 ± 0.86^{abc}	6.03 ± 0.84^{de}

*Different superscript letters indicate significant differences among treatments and seasons ($p < 0.05$). Data shown are means ± SE. CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

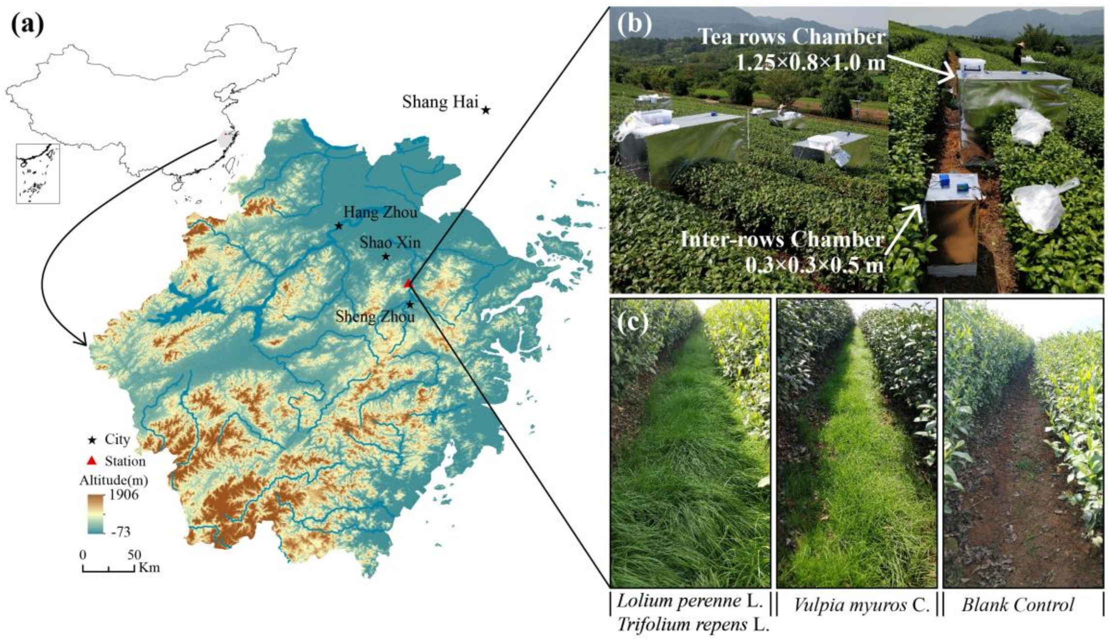

Figure 1. (a) Geographic location of the study area in Shengzhou City, Zhejiang Province, China; (b) field layout of the tea plantation experiment; (c) photos of *Lolium perenne* L. and *Trifolium repens* L. plot, and *Vulpia myuros* C. plot, the blank control plot, respectively.

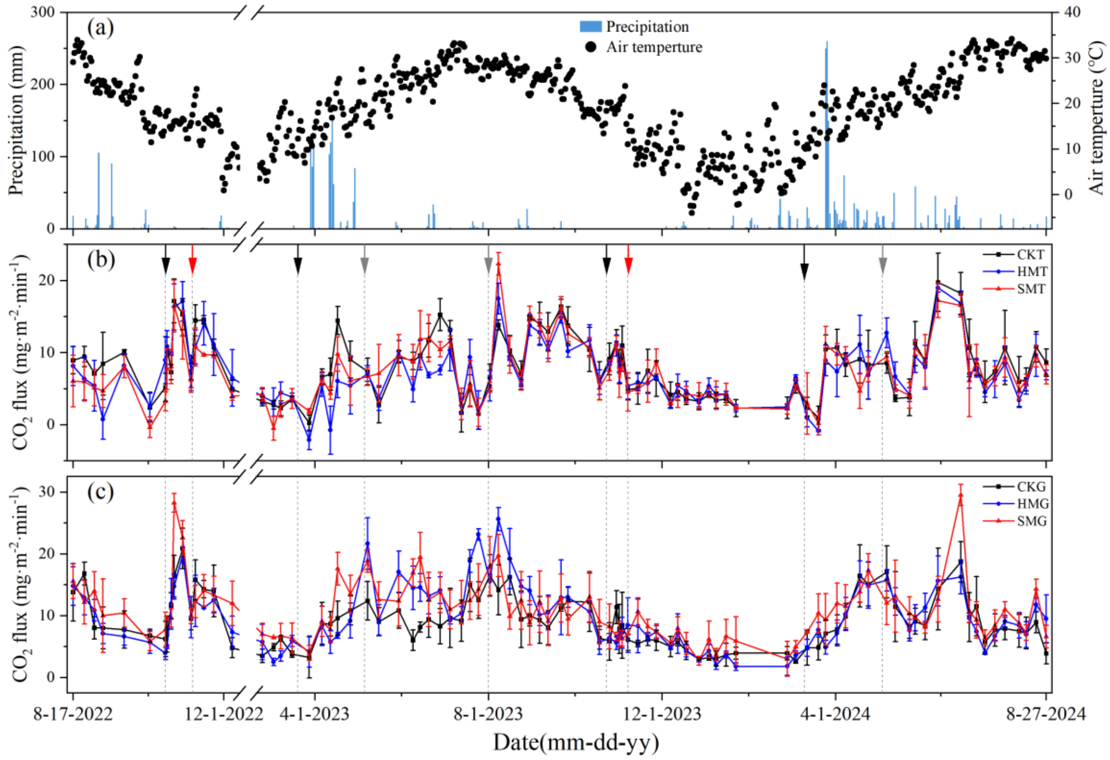

Figure 2. Dynamics of (a) air temperature and precipitation, (b) CO_2 fluxes from tea rows, and (c) CO_2 fluxes from inter-rows during the observation period (2022–2024). Black, green and orange arrows represent the timings of fertilization, grass planting and tea pruning, respectively. Flux data are presented as mean ± SE. CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

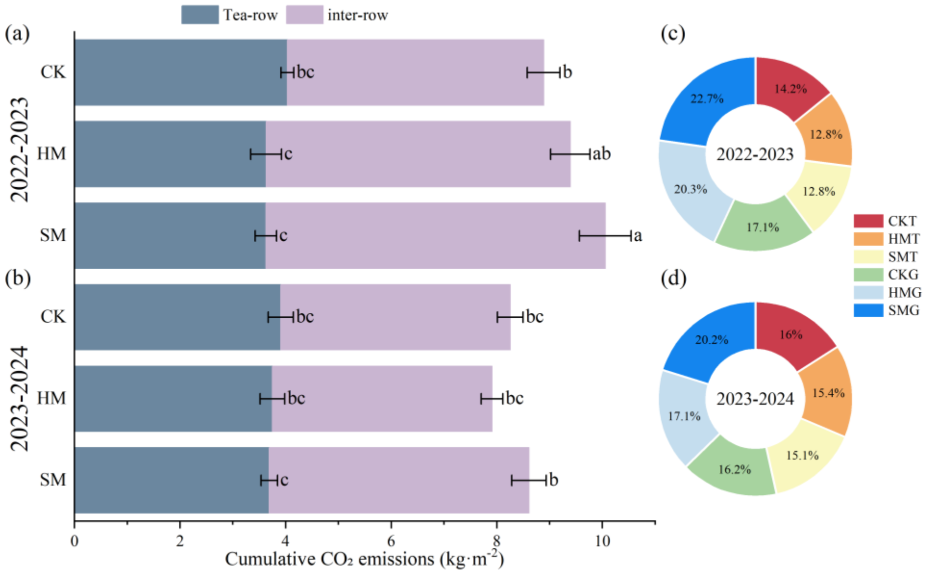

Figure 3. (a, b) Annual cumulative CO_2 emissions from tea rows and inter-rows under different green manure intercropping treatments; (c, d) contribution of tea rows and inter-rows to total annual CO_2 emissions under each treatment. Data shown are means ± SE. Different superscript letters denote statistically significant differences ($p < 0.05$). CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

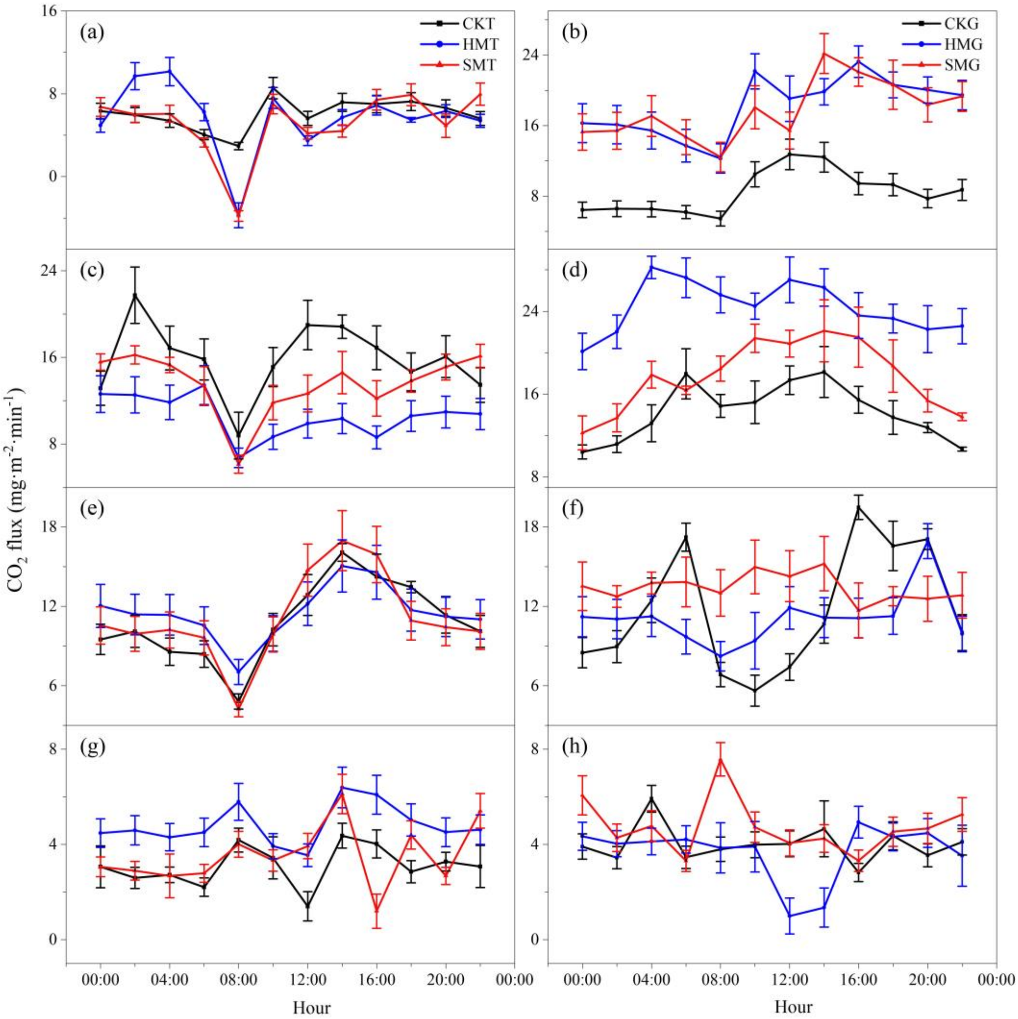

Figure 4. Diurnal variation in CO_2 fluxes from (a, c, e, g) tea rows and (b, d, f, h) inter-row zones under different green manure intercropping treatments across seasons: (a–b) spring, (c–d) summer, (e–f) autumn, and (g–h) winter. CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

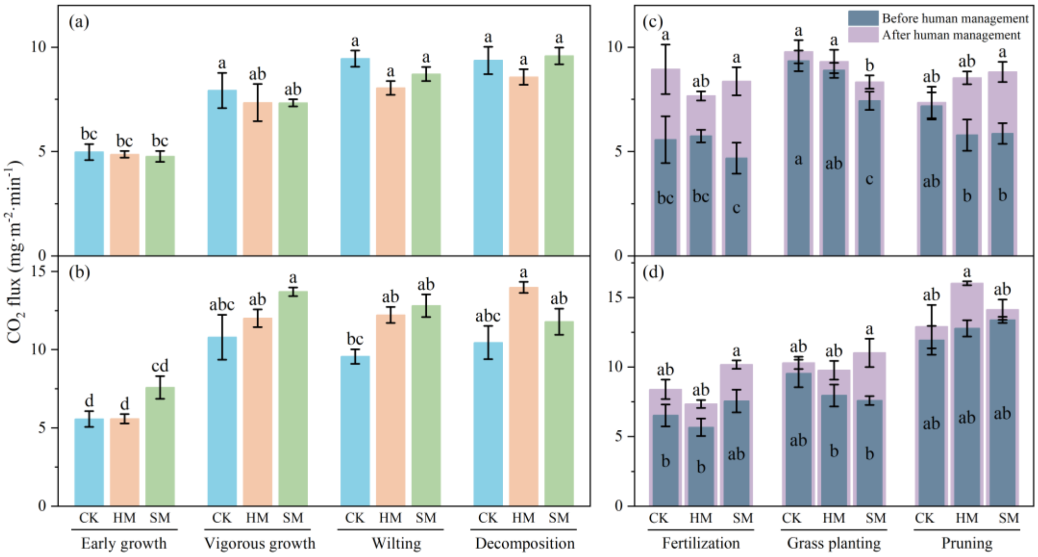

Figure 5. Temporal dynamics of CO_2 fluxes under green manure (a, b) growth stages and (c, d) management events in tea plantations. Growth stages include: early growth (mid-November to early April), vigorous growth (mid-April to late May), wilting (early June to late July), and decomposition (August). CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

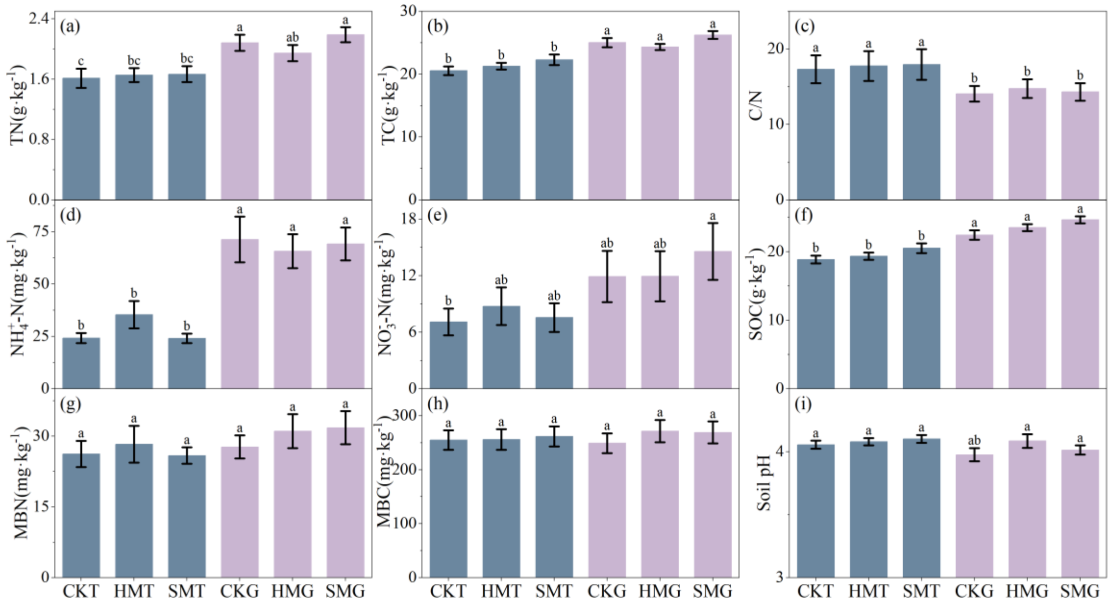

Figure 6. Basic physicochemical properties of soil in tea rows and inter-rows under different green manure intercropping treatments. CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

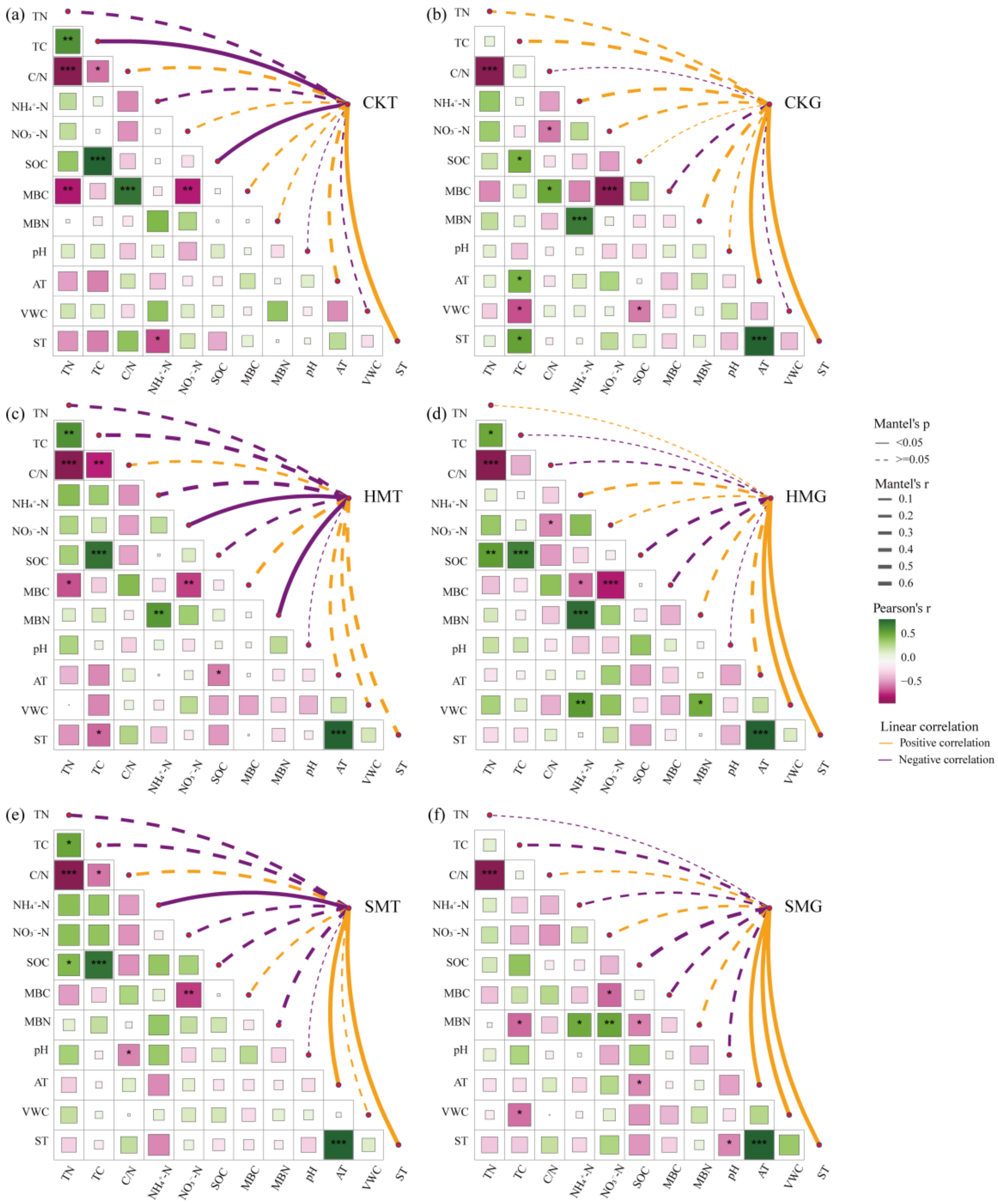

Figure 7. Pairwise correlations between environmental factors and their relationships with CO_2 fluxes under different green manure treatments (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

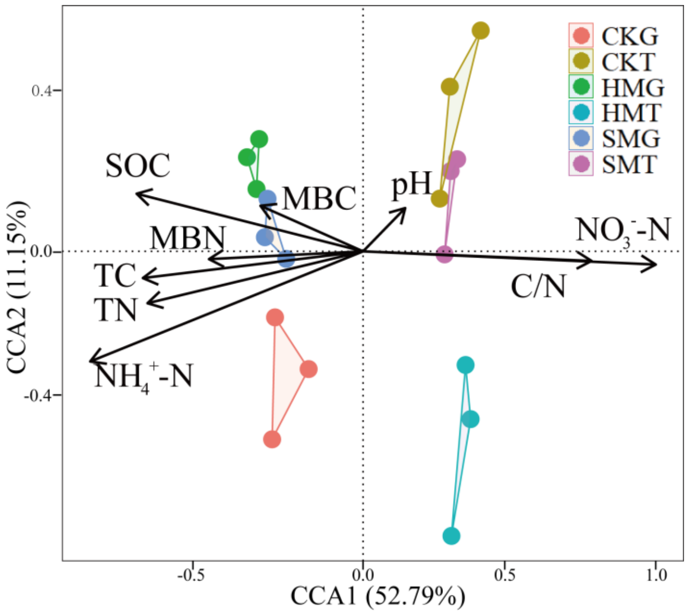

Figure 8. Canonical correspondence analysis (CCA) showing the influence of soil physicochemical properties on CO_2 emissions from tea rows and inter-rows under different green manure treatments. CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.

Appendix 1

The CO_2 flux was calculated using the following equation:

$$F = \rho_0 \times \frac{P}{P_O} \times \frac{T_O}{T+T_O} \times \frac{V}{M} \times \frac{\Delta c}{\Delta t} \quad (1)$$

where F is the CO_2 flux ($mg \cdot m^{-2} \cdot min^{-1}$); ρ_0 is the density of CO_2 under standard conditions ($1.98\ kg \cdot m^{-3}$); P_O and T_O are the standard atmospheric pressure (101.325 kPa) and temperature (273.15 K), respectively; P and T are the atmospheric pressure (kPa) and absolute temperature (K) at the time of sampling; V and M are the volume (m^3) and bottom area (m^2) of the chamber, respectively; $\triangle c/\triangle t$ is the slope of the linear or nonlinear regression of CO_2 concentration over time.

First, the raw CO_2 concentration readings were calibrated with standard gases. Then, linear regression was performed to fit their rate of change over time. Finally, CO_2 flux was calculated using the standard flux formula by incorporating the chamber volume, base area, and the measured atmospheric pressure and temperature.

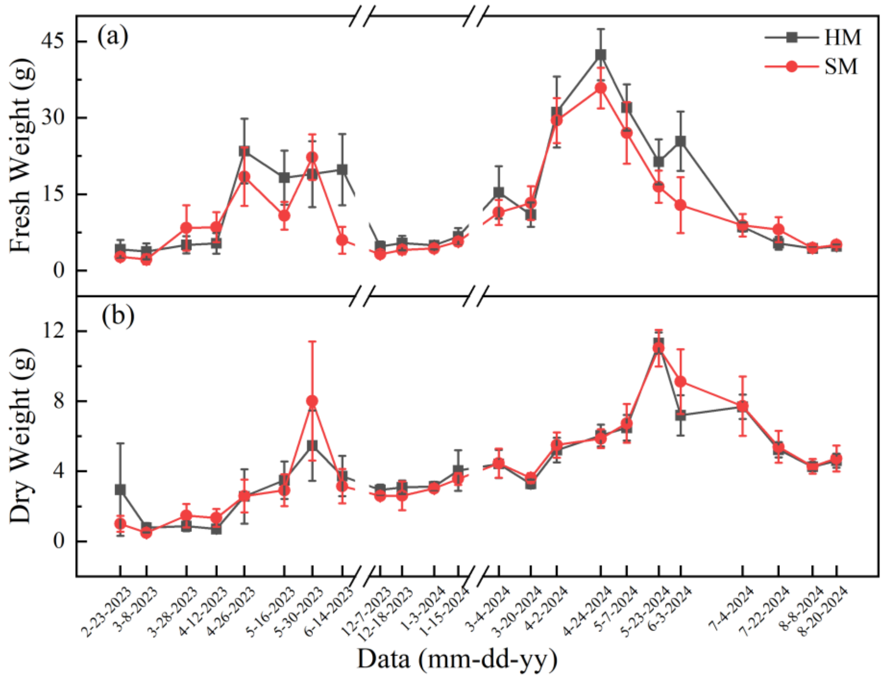

Figure A1. Temporal trend of green manure indexes