

Revision EGUSPHERE-2025-5065

Point by point response to the reviewers:

First of all, we would like to thank the editor and 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

This study focuses on a subtropical tea plantation and utilizes the static chamber–gas chromatography method to conduct long-term monitoring of CO₂ emission fluxes under different green manure intercropping treatments. It systematically reveals the short- and long-term trends and differences in CO₂ fluxes across different areas of the tea garden, including between tea rows, and analyzes the impact of key environmental drivers such as soil temperature, moisture, and organic carbon on carbon emissions. The findings provide important insights into the carbon cycling mechanisms in tea plantations under green manure intercropping and hold significant practical value for promoting low-carbon management in tea cultivation. The experimental design is reasonable, the analysis and discussion are thorough, and the structure is clear, making the paper suitable for publication in this journal. The following minor revisions are recommended:

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:

1. Lines 153–154: The baseline physicochemical properties of the soil in the study area are not provided. It is recommended to include initial soil characteristics.

Response: Thank you for the comments. We have supplemented a detailed table of the basic physicochemical properties of the six treatment soils before the experiment (now Table 1) in Sec. 2.1 Lines 164–166.

“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. Line 171: In addition to the green manure treatment, please specify the type and application rate of fertilizers used.

Response: Thank you for your comment. Regarding the fertilizer treatments mentioned on line 178-180, the specific types and application rates are as follows:

On October 21, 2022, rapeseed cake was applied as base fertilizer at a rate of 300 kg per mu after trenching and soil turning between the tea rows. On October 28, 2023, base fertilizer consisting of rapeseed cake (65 kg per mu) and compound fertilizer (25 kg per mu) was applied following trenching and soil turning in the tea row inter-rows.

3. Line 185: For consistency, please express time in 24-hour format. Replace “between 9:00 and 11:00 a.m.” with “between 09:00 and 11:00 (local time).”

Response: Thank you for the comments. We revised it (Line 192).

4. Line 198: What was the soil sampling depth? This significantly influences soil physicochemical properties. Sampling across multiple soil layers would be more appropriate.

Response: Thank you for the suggestion. The soil sampling depth was 0–20 cm. The core focus of this study is the impact of green manure treatments on soil carbon processes in tea plantations. Since the root systems of green manure and their incorporation primarily concentrate in the 0–20 cm soil layer, sampling at this depth most directly reflects the treatment effects. To avoid destructive deep-layer sampling that could harm the root systems of productive tea plants and to ensure the continuity of long-term observations, this biologically active layer was selected as the representative depth, which is also a commonly adopted standard in comparable studies both domestically and internationally. We recognize the value of stratified sampling and will aim to refine this aspect in future research.

5. Lines 216–217: The method for calculating CO₂ fluxes is not clearly described. Please provide details.

Response: Thank you for the comments. The description of the CO₂ flux calculation method has been added in the Appendix 1.

6. Lines 308–310: On what basis were the growth stages of green manure defined? Please clarify.

Response: Thank you for the comments. The growth stages of green manure are divided as follows: early growth stage (from mid-November to early April of the following year, primarily functioning for soil surface coverage and water-soil conservation), vigorous growth stage (from mid-April to late May, when biomass and nutrient retention peak), wilting stage (from early June to late July, as the plants naturally wither and prepare for incorporation), and decomposition stage (during August each year, when residues rapidly decompose and release nutrients). This division is based on field phenological observations and biomass dynamics, clarifying the functional transition of each stage within the tea garden ecosystem—from growth and accumulation to incorporation and decomposition—to precisely align with the nutrient management needs of tea garden soils.

7. Lines 498–499: The decrease in cumulative emissions between rows in the second year is attributed to “reduced human disturbance,” which is insufficiently supported. Please elaborate with references to relevant literature.

Response: Thank you for your comments. Yes, attributing the decrease in CO₂ emissions simply to "reduced anthropogenic disturbance" is overly general. In this study, the cumulative CO₂ emissions from the inter-row areas in the second year were lower than those in the first year across all treatments, with a more pronounced reduction observed in the green manure treatments (HM, SM). We believe this change is closely related to the gradual attenuation of the priming effect caused by initial soil disturbance and the continuous improvement in soil structure

facilitated by green manure growth. We added more discussion in Sec. 4.2 (Lines 513–523).

“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 CO₂ emissions observed in the following year.”

8. Line 541: The discussion on the SOC threshold lacks adequate references. Additional literature should be cited and discussed.

Response: Thank you for the comments. We added more discussion in Sec. 4.3 (Lines 569–574).

“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 NO₃[−]-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).”

9. Table 1: Please clearly note “Values are mean ± SE” in the table caption or footnote.

Response: Thank you for the comments. We noted “Data shown are means ± SE.” in the table 1 footnote.

10. Root biomass data are lacking. The contribution of green manure roots to soil respiration has not been quantified, which may affect the interpretation of CO₂ flux sources.

Response: Thank you for your important comment. We have added Figure A1 in Appendix 1, which presents the green manure biomass (including above ground parts and root systems) for the HM and SM treatments. The biomass was measured as fresh weight immediately after sampling and as dry weight after oven-drying at 65°C to constant weight. We added more discussion in Sec. 4.2 (Lines 517–523).

“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 CO₂ emissions observed in the following year.”

11. Within the closed chamber environment, temperature and humidity change over time, potentially influencing CO₂ flux measurements. Further analysis on this aspect is recommended.

Response: Thank you for the comments. The static chamber method has an inherent limitation: changes in the chamber micro-environment (temperature, humidity, air pressure) during measurement may affect the calculated CO₂ flux. To minimize this impact, the following measures were taken in this experiment:

1. Controlled measurement duration: The chamber closure time was strictly limited to 21 minutes for each measurement to reduce accumulated deviation in the chamber environment.
2. Standardized operation: Measurements were conducted during periods of stable weather. A fan was installed inside the chamber to mix the air, while the exterior was wrapped with aluminum foil and sponge to prevent rapid internal temperature

rise due to direct sunlight during sampling.

3. Linear relationship verification: We ensured that the CO₂ concentration showed a strong linear relationship with time within the selected measurement window ($R^2 > 0.95$), indicating that changes in respiration rate due to chamber environmental variations were not significant during the measurement period.

12. The study does not explore how different green manure treatments regulate microbial activity or the mechanisms by which soil microbial communities drive carbon sequestration. If relevant measurements were not included, this could be addressed in future research.

Response: Thank you for the comments. We fully agree that elucidating how green manure treatments drive soil carbon sequestration by regulating microbial activity and community structure is a crucial component for a complete explanation of the ecological mechanisms involved. The current study primarily focuses on the response relationships among green manure, soil physicochemical properties, and CO₂ emission fluxes. Due to limitations in research design and duration, it did not include measurements related to microbial community structure and function. Follow-up research will place greater emphasis on investigating the role of soil microorganisms.

13. The language throughout the manuscript should be further refined to avoid repetitive statements, particularly in the Results and Discussion sections.

Response: Thank you for the comments. We have refined the language throughout the manuscript to improve conciseness and eliminate repetitive statements. The revisions have focused on consolidating redundant descriptions, employing more precise and varied phrasing, and strengthening the logical flow of the argument.

1 **Spatially Contrasting CO₂ Dynamics Driven by Green Manure**
2 **Intercropping in Subtropical Tea Plantations**

3
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19 **Abstract:**

20 Tea plantations are important contributors to greenhouse gas emissions due to intensive
21 fertilization and continuous cultivation. However, the mechanisms by which green
22 manure intercropping regulates soil CO₂ dynamics in these systems remain poorly
23 understood. We employed the static chamber method over a two-year period, with
24 sampling conducted weekly, to investigate ~~We investigated~~ how intercropping with
25 *Vulpia myuros* (SM) and a legume–nonlegume mixture of *Lolium perenne* and *Trifolium*
26 *repens* (HM) influenced spatial CO₂ flux dynamics compared with a no-intercropping
27 control (CK) from tea rows and inter-row zones in a subtropical tea plantation. Distinct
28 seasonal variations were observed, with CO₂ fluxes peaking in summer and autumn and
29 declining in spring and winter. ~~Average tea-row fluxes were 7.41 ± 0.45 , 7.35 ± 0.44 ,~~
30 ~~and 8.12 ± 0.46 mg·m⁻²·min⁻¹ under SM, HM, and CK, respectively, indicating~~
31 ~~emission reductions with intercropping. In contrast, inter-row fluxes were higher under~~
32 ~~SM (10.83 ± 0.52 mg·m⁻² min⁻¹) and HM (9.77 ± 0.54 mg·m⁻² min⁻¹) than under CK~~
33 ~~(9.07 ± 0.44 mg·m⁻² min⁻¹), demonstrating pronounced spatial contrasts. Average tea-~~
34 ~~row fluxes were 8.7% and 9.5% lower under SM and HM, respectively, compared to~~
35 ~~CK, indicating emission reductions with intercropping. In contrast, average inter-row~~
36 ~~fluxes increased by 19.4% under SM and 7.7% under HM, demonstrating pronounced~~
37 ~~spatial contrasts. Diurnal patterns exhibited midday peaks (12:00–14:00), especially in~~
38 ~~spring and summer, and short-term CO₂ pulses were triggered by field operations such~~
39 ~~as fertilization and pruning. Diurnal patterns generally exhibited midday peaks (12:00–~~
40 ~~14:00), especially in summer and autumn across all tea-rows, and short-term CO₂ pulses~~
41 ~~were triggered by field operations such as fertilization and pruning.~~ Notably, HM
42 effectively suppressed fertilization-induced CO₂ pulses, revealing the mitigation
43 potential of legume–nonlegume mixtures. Green manure increased soil organic carbon
44 (6.4%), lowered soil temperature (4.5%), and enhanced porosity (4.2%), collectively
45 shaping CO₂ dynamics. Multivariate analysis identified soil organic carbon (SOC) and
46 temperature as dominant flux drivers, and a potential SOC threshold was detected,
47 beyond which CO₂ emissions accelerated. Compared to CK, While intercropping
48 reduced tea-row emissions by 7.1–7.9% but increased inter-row emissions by 12.7–

49 28.9%₅ based on the two years cumulative emissions, continuous intercropping
50 significantly decreased overall inter-row emissions over time. These results highlight
51 the spatially heterogeneous nature of carbon flux regulation and demonstrate the long-
52 term potential of green manure intercropping as a climate-smart management strategy
53 in perennial agroecosystems.

54

55 **Keywords:** tea plantations, green manure, CO₂ emissions, soil factors

56 **1 Introduction**

57 Mitigating greenhouse gas (GHG) emissions to address global warming and
58 associated climate challenges remains a priority in global environmental research.
59 Among long-lived GHGs, carbon dioxide (CO₂) plays the most prominent role,
60 contributing approximately 66% to the increase in global radiative forcing (IPCC,
61 2022). In 2023, the global average atmospheric CO₂ concentration reached 420.0 ± 0.1
62 ppm, representing a 151% increase compared to pre-industrial levels (prior to 1750)
63 (WMO, 2024). Agriculture is a major emission sector, accounting for about 14% of
64 total anthropogenic CO₂ emissions (Wang et al., 2025). In China, this share is even
65 higher, with agricultural activities accounting for up to 17% of national CO₂ emissions
66 (Xu and Lin, 2017). Therefore, accurately characterizing CO₂ emission dynamics in
67 agricultural systems and scientifically informed mitigation strategies are critical for
68 advancing global GHG reduction efforts and promoting sustainable, low-carbon
69 agricultural development (Xu et al., 2024).

70 Tea (*Camellia sinensis* L.) is an important economic crop in tropical and
71 subtropical regions. Over recent decades, global tea cultivation area has expanded
72 rapidly, reaching 4.70 million hectares in 2022. China has led the most significant
73 growth, with 3.35 million hectares of tea plantations and an annual production of 2.82
74 million tons, ranking first worldwide in both area and output (FAO, 2024). To maximize
75 yield and improve tea quality, fertilizer inputs to tea cultivation area can be up to four
76 times higher than those applied to staple crops during a single growing season (Zou et
77 al., 2009; Han et al., 2013; Yao et al., 2015). In China, average annual fertilizer use in
78 tea plantations reaches 678 kg ha⁻¹, with more than 30% of plantations experiencing
79 over-application (Ni et al., 2019). Such intensive fertilization not only accelerates soil
80 acidification but also significantly increases GHGs emissions from tea plantations (Liu
81 et al., 2016; Yan et al., 2020). However, most existing studies on agricultural CO₂
82 emissions have focused on staple cropping systems such as wheat (Song et al., 2024),
83 rice (Qian et al., 2023), and maize (Zhang et al., 2020), while studies on CO₂ emissions
84 from tea plantations remain limited.

85 For instance, Lang et al. (2017) reported that intercropping rubber trees with tea

86 in the tropical forests of Xishuangbanna, China, reduced CO₂ emissions, although it
87 simultaneously weakened the soil's methane (CH₄) uptake capacity. Wanyama et al.
88 (2019) found that converting tropical montane forests into tea plantations in Africa
89 decreased annual soil CO₂ emissions to 5.6 t ha⁻¹, with emissions positively correlated
90 with soil pH and negatively correlated with the soil C/N ratio. Pang et al. (2019)
91 quantitatively assessed the net ecosystem exchange (NEE) of tea plantations in
92 southeastern China from 2014 to 2017, reporting values ranging from -182.40 to
93 -301.51 g C m⁻², indicating that tea plantations act as net carbon sinks. However, their
94 carbon sequestration potential was lower than that of other subtropical ecosystems, with
95 temperature identified as the primary factor influencing ecosystem respiration. These
96 findings suggest that CO₂ emissions from tea plantations play a non-negligible role on
97 the carbon exchange between atmosphere and tea plantations. However, the limited
98 number of studies has led to substantial uncertainty on estimating tea plantation CO₂
99 emissions, restricting our understanding of their contribution to regional and global
100 agricultural GHG budgets (Li et al., 2016; Ji et al., 2020) and hindering the development
101 of low-carbon tea plantations.

102 In response, there is a growing emphasis on the development of eco-friendly and
103 low-carbon tea plantations (Wang et al., 2022). Toward reduction of fertilizer usage and
104 higher economic efficiency, various management practices were incorporated,
105 including by using green manure. As a modernized agricultural practice, green manure
106 has been widely adopted in farming systems and serves as an important measure for
107 improving soil quality, playing a vital role in sustainable agriculture. Within the context
108 of GHGs mitigation, green manure is recognized as an effective solution for improving
109 soil quality and enhancing CO₂ sequestration in agroecosystems (Forte et al., 2017).
110 However, most studies examining the relationship between green manure and carbon
111 emissions have focused on conventional croplands such as rice and wheat.
112 Comprehensive studies have shown that appropriate green manure management can
113 significantly reduce the global warming potential (GWP) associated with fertilization
114 (Zhang et al., 2024). For instance, Gong et al. (2021) demonstrated that long-term
115 ryegrass cover in organic soybean fields effectively reduced net GWP. In contrast, other

116 studies have reported that green manure application may increase CO₂ emissions. Kim
117 et al. (2013) found that the application of Chinese milk vetch and ryegrass increased
118 winter CO₂ fluxes in paddy fields by approximately 197% and 266%, respectively.
119 Large-scale assessments have further revealed that green manure tends to increase CO₂
120 emissions, primarily due to differences in plant species and biomass inputs. Biomass
121 alone explained 63% of the variation in CO₂ emission increases, with emissions
122 declining as the C/N ratio of cover crop biomass increased. Notably, mixed sowing of
123 leguminous and non-leguminous green manures has been shown to improve residue
124 C/N ratios and reduce GHGs emissions (Muhammad et al., 2019).

125 Existing studies on GHG mitigation in tea systems have predominantly focused on
126 fertilizer reduction and substitution strategies. Wu et al. (2018) conducted a three-year
127 fertilization control experiment in southern China and found that halving nitrogen input
128 decreased nitrous oxide (N₂O) emissions by 44.5% in tea plantations. In contrast, Yao
129 et al. (2015) reported that organic fertilizer application led to a 71% increase in N₂O
130 emissions compared to conventional urea, suggesting potential trade-offs in GHG
131 outcomes. Organic amendments, such as compost or manure, have been shown to
132 improve soil fertility, enhance soil structure, porosity, and pH, and promote carbon
133 sequestration in tea plantation soils (Han et al., 2013; Wu et al., 2021). Biochar
134 application has also been identified as an effective strategy for improving soil quality
135 while simultaneously enhancing soil carbon storage and reducing emissions (Wu et al.,
136 2021). The effect of green manure intercropping on tea plantations was mainly focused
137 on improvements in tea plant growth and soil nutrient dynamics. For example,
138 intercropping with green manure species has been shown to enhance nitrogen use
139 efficiency and increase soil microbial diversity (Huang et al., 2023). The potential role
140 of green manure intercropping in mitigating GHGs emissions in tea ecosystems remains
141 poorly understood, and its interactions with key environmental factors have not been
142 fully clarified (Zhu et al., 2018). Green manure may influence CO₂ emissions by
143 altering carbon input levels and inducing soil disturbances, but the specific emission
144 characteristics and driving factors require further investigation.

145 To address these gaps, this study selected cultivated tea plantations region located

146 in the east of China, where is recognized as a very important tea cultivation area famous
147 by the tea name of Longjing. Commonly used green manure species in tea systems
148 (*Vulpia myuros* C., *Lolium perenne* L., and *Trifolium repens* L.) were selected for
149 intercropping, covering both leguminous and non-leguminous species, under
150 monoculture and mixed-sowing configurations. CO₂ flux were carried out in both tea
151 rows and inter-row zones across different intercropping treatments. Dynamics and key
152 influencing factors of CO₂ emissions under various green manure intercropping models
153 were analyzed, aiming to reveal the potential reasons by which green manure
154 intercropping regulates carbon fluxes in tea plantations and provide support for the
155 development of low-carbon tea plantations and sustainable regional agriculture.

156

157 **2 Methodology**

158 **2.1 Monitoring Site**

159 This study was conducted at the Comprehensive Experimental Tea Plantation Base
160 of the Tea Research Institute, Chinese Academy of Agricultural Sciences, located in
161 Shengzhou, Zhejiang Province, China (120°83'E, 29°75'N; elevation 30 m a.s.l.) (Fig.
162 1a). The site is situated in a low mountainous and hilly region of southeastern China
163 and is characterized by a subtropical monsoon climate. During the experimental period
164 (August 2022 to August 2024), the average annual temperature was approximately
165 16 °C, with an average annual precipitation of about 1400 mm. The region experiences
166 a concentrated rainy season from April to June and has a frost-free period of around
167 240 days. The tea cultivated at the site is *Jinmudan*, an elite cultivar derived from the
168 hybridization of *Tieguanyin* and *Huangdan*, and is widely planted across China. The
169 tea plantation was established in 2015; the tea plants are 8–10 years old and arranged
170 in a single-row planting pattern with a row spacing of 150 cm and a plant spacing of 40
171 cm. The soil type of the tea plantation is classified as red soil (Ultisol), typical soil
172 species in this region. The soil type of the tea plantation is classified as red soil (Ultisol),
173 a typical soil type in this region, and the initial basic physicochemical properties of the
174 six treatment soils are shown in Table 1.

175

176 **2.2 Experimental Setup**

177 Three green manure intercropping treatments were established in this study: *Vulpia*
178 *myuros* C. (SM), a mixture of *Lolium perenne* L. and *Trifolium repens* L. (HM), and a
179 control treatment without intercropping (CK). The experimental tea plantation covered
180 an area of approximately 1000 m². For each treatment, three representative tea rows
181 were selected as three replicates. Adjacent treatments were separated by two tea rows
182 (~3 m), and replicate areas within the same treatment were spaced approximately 5 m
183 apart. Each treatment has three duplications. Gas fluxes were measured in both tea rows
184 (T) and inter-row zones (G), resulting in six treatments: SMT, SMG, HMT, HMG, CKT,
185 and CKG, with a total of 18 representative sampling points (Fig. 1b, c). Tea plantation
186 followed standard management practices, including fertilization, pruning, and tillage.
187 Basal fertilization and tillage were conducted in middle of October, followed by green
188 manure sowing in early November. On October 21, 2022, rapeseed cake was applied as
189 base fertilizer at a rate of 2250 kg ha⁻¹ after trenching and soil turning between the tea
190 rows. On October 28, 2023, base fertilizer consisting of rapeseed cake (975 kg ha⁻¹) and
191 compound fertilizer (375 kg ha⁻¹) was applied following trenching and soil turning in
192 the tea row inter rows. Urea was applied as a topdressing in early February. Tea leaves
193 were harvested in end of March, and pruning was normally conducted in May and July.

194 Gas sampling was performed using the static chamber-gas chromatography
195 method. The dimensions of the static chambers were 1.25 × 0.8 × 1.0 m for tea rows and
196 0.3 × 0.3 × 0.5 m for inter-row zones (Fig. 1b). Each chamber was equipped with an
197 internal fan to ensure uniform gas mixing. To avoid rapid heating due to sunlight, the
198 chambers were wrapped with aluminum foil and sponge, functioning as dark chambers.
199 To minimize disturbance, chamber bases with water grooves were installed one month
200 in advance at each sampling point, inserted 15 cm into the soil. During sampling, water
201 was added to the grooves, and the chamber was securely sealed onto the base to create
202 a closed environment. Four gas samples were collected at 7-minute intervals using gas
203 sampling bags.

204 For seasonal monitoring, sampling was conducted once per week between 09:00
205 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). CO₂ 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 CO₂ 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 (R²) 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 (NO₃⁻-N) and ammonium nitrogen (NH₄⁺-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*

236 using a portable soil sensor (TDR-315H, Acclima). Soil bulk density (BD) and water-
237 filled pore space (WFPS) were determined using the core ring method.

238

239 **2.3 Data Processing**

240 The flux refers to the amount of gas exchanged per unit time and unit area. A
241 positive value indicates net emission to the atmosphere, while a negative value indicates
242 net uptake from the atmosphere (Yao et al., 2015; Zhang et al., 2020). Based on the flux
243 measurements, cumulative CO₂ emissions under different green manure intercropping
244 treatments were also estimated. All data analyses and visualizations were performed
245 using R software. Two-way analysis of variance (Two-way ANOVA) was employed to
246 assess the effects of treatment type and observation period on CO₂ fluxes and soil
247 physicochemical properties. Spearman correlation analysis and Mantel tests were used
248 to examine the relationships between CO₂ flux and environmental variables under
249 different green manure intercropping treatments. Canonical correspondence analysis
250 (CCA) was applied to comprehensively evaluate the influence of soil physicochemical
251 properties on CO₂ emissions. Data shown are means \pm standard error (SE). In all
252 statistical tests, the level of significant differences and correlations was set at $p < 0.05$.

253

254 **3 Results**

255 **3.1 Long-term Variation of CO₂ Fluxes under Green Manure Intercropping**

256 Figure 2 illustrates the long-term trends of key environmental variables and CO₂
257 fluxes in the tea plantation throughout the observation period. Overall, CO₂ fluxes from
258 both tea-row and inter-row zones displayed distinct seasonal patterns: higher in summer
259 and autumn, and lower in spring and winter. The seasonal differences between the warm
260 (summer and autumn) and cool (spring and winter) periods were statistically significant
261 (Table 42). The temporal dynamics of CO₂ fluxes closely tracked the trends in air
262 temperature (Fig. 2a), suggesting that temperature is a key driver of soil respiration in
263 tea plantations. Annual fluctuations in CO₂ fluxes were also strongly influenced by field
264 management activities. For example, a sharp increase in CO₂ emissions was observed
265 following basal fertilization in October, and another rise occurred in March of the

266 following year after topdressing and with the onset of warmer temperatures, ultimately
267 peaking in summer (Fig. 2b-c). The effects of management activities are further detailed
268 in Section 3.3.

269 In tea rows, the annual mean CO_2 fluxes under HMT and SMT treatments were
270 $7.35 \pm 0.44 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ and $7.41 \pm 0.45 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$, respectively, both lower than
271 that of the control (CKT: $8.12 \pm 0.46 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) (Fig. 2b). In contrast, in inter-row
272 zones, the annual mean CO_2 fluxes were significantly higher under HMG (9.77 ± 0.54
273 $\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) and SMG ($10.83 \pm 0.52 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) compared to the control (CKG:
274 $9.07 \pm 0.44 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) ($p < 0.05$) (Fig. 2c). Across seasons, CKT generally
275 exhibited higher CO_2 fluxes than HMT and SMT, except during winter. In the inter-row
276 zones, both HMG and SMG showed significantly higher fluxes than CKG in summer,
277 while SMG consistently had significantly higher CO_2 emissions than both CKG and
278 HMG during the remaining seasons ($p < 0.05$) (Table 42).

279 Overall, green manure intercropping significantly increased CO_2 emissions from
280 inter-rows, but reduced emissions in tea rows. In terms of cumulative annual emissions,
281 HMT and SMT resulted in $3.69 \text{ kg} \cdot \text{m}^{-2}$ and $3.66 \text{ kg} \cdot \text{m}^{-2}$ of CO_2 emissions, respectively,
282 both lower than the $3.97 \text{ kg} \cdot \text{m}^{-2}$ under CKT (Fig. 3). Similarly, cumulative CO_2
283 emissions under HMG and SMG remained consistently higher than under CKG, but
284 they declined from $5.76 \text{ kg} \cdot \text{m}^{-2}$ and $6.43 \text{ kg} \cdot \text{m}^{-2}$ in the first year to $4.16 \text{ kg} \cdot \text{m}^{-2}$ and 4.92
285 $\text{kg} \cdot \text{m}^{-2}$ in the second year, respectively (Fig. 3). Two consecutive years of green manure
286 intercropping led to a gradual reduction in CO_2 emissions from inter-rows, indicating
287 its potential role in long-term emission mitigation in tea plantations. CO_2 emissions
288 from inter-rows were substantially higher than those from tea rows. Compared with the
289 control, HM and SM intercropping increased inter-row cumulative CO_2 emissions by
290 12.7% and 28.9%, respectively, while reducing tea-row emissions by 7.1% and 7.9%
291 (Fig. 3a-b). Inter-row zones accounted for 52.6%, 57.3%, and 60.8% of the total annual
292 CO_2 emissions in the CK, HM, and SM treatments, respectively (Fig. 3c-d), indicating
293 that the inter-row emissions cannot be ignored.

294

295 **3.2 Diurnal CO₂ Variations**

296 CO₂ fluxes in the tea plantation exhibited pronounced diurnal variations across all
297 seasons, particularly in spring and summer (Fig. 4), likely influenced by the growth
298 stages of green manure species. In spring, CO₂ fluxes in tea rows under all treatments
299 showed a similar diurnal trend: an initial decline followed by a rapid increase. HMT
300 and SMT reached their minimum fluxes at 08:00 (local time), with values of -3.74
301 mg·m⁻²·min⁻¹ and -3.80 mg·m⁻²·min⁻¹, respectively, then rose sharply and stabilized in
302 the afternoon. The diurnal amplitudes under HMT and SMT were notably greater than
303 that of the control (CKT) (Fig. 4a). In the inter-row zones, the diurnal patterns under
304 green manure treatments differed notably from the control (Fig. 4b). CKG displayed a
305 unimodal pattern with a peak at 12:00 (12.74 mg·m⁻²·min⁻¹) and a trough at 08:00 (5.45
306 mg·m⁻²·min⁻¹), resulting in an amplitude of 7.29 mg·m⁻²·min⁻¹. In contrast, HMG and
307 SMG exhibited later peaks at 16:00 (23.26 mg·m⁻²·min⁻¹) and 14:00 (24.17
308 mg·m⁻²·min⁻¹), respectively, with troughs also at 08:00 (HMG: 12.28 mg·m⁻²·min⁻¹;
309 SMG: 12.43 mg·m⁻²·min⁻¹). Both treatments showed substantially higher amplitudes
310 than CKG.

311 Summer exhibited the most pronounced diurnal variation of CO₂ fluxes across all
312 seasons. In tea rows, CKT, HMT, and SMT followed a bimodal pattern, with peaks at
313 02:00 and 12:00, and a trough at 08:00. Their respective diurnal amplitudes were 12.96 ,
314 6.70 , and 10.10 mg·m⁻²·min⁻¹ (Fig. 4c). In the inter-rows, the amplitudes were
315 relatively lower, 7.72 , 8.12 , and 7.79 mg·m⁻²·min⁻¹ for CKG, HMG, and SMG,
316 respectively, indicating smaller fluctuations compared to tea rows (Fig. 4d). Notably,
317 summer also showed the most distinct contrast between tea rows and inter-rows: CKT
318 recorded the highest average flux in the tea rows, while CKG had the lowest in the inter-
319 rows.

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

325 SMG (Fig. 4f).

326 In winter, CO₂ fluxes showed the most stable diurnal variation of the year. In tea
327 rows, amplitudes were only 2.96, 2.84, and 4.92 mg·m⁻²·min⁻¹ for CKT, HMT, and
328 SMT, respectively (Fig. 4g). Unlike other seasons, 08:00 no longer corresponded to the
329 daily minimum but rather to a relative maximum, with daily peaks generally occurring
330 at 14:00. In inter-rows, diurnal patterns were less defined. SMG exhibited the highest
331 flux at 08:00 (7.57 mg·m⁻²·min⁻¹), while HMG showed the lowest at 14:00 (0.99
332 mg·m⁻²·min⁻¹) (Fig. 4h).

333

334 **3.3 Effect of Human Management on CO₂ Fluxes**

335 CO₂ fluxes from the tea field varied significantly across different growth stages of
336 green manure, exhibiting a general increasing trend from the early growth stage to the
337 vigorous, wilting, and decomposition stages (Fig. 5a). In the tea rows, the lowest fluxes
338 were observed during the early growth stage, while the highest occurred during the
339 decomposition stage. Differences among the three treatments (CKT, HMT, and SMT)
340 were minimal during the early growth but became more apparent in the subsequent
341 stages. Notably, during the vigorous stage, both HMT and SMT treatments reduced CO₂
342 emissions compared to CKT. In contrast, the impact of green manure growth on CO₂
343 fluxes was more pronounced in the inter-row zones (Fig. 5b). At all growth stages, CO₂
344 fluxes under the HMG and SMG treatments were significantly higher than those under
345 CKG, with the largest differences observed during the wilting stage. Peak emissions
346 occurred during the decomposition stage for HMG and during the wilting stage for
347 SMG.

348 Fertilization substantially increased CO₂ emissions across the tea plantation (Fig.
349 5c-d). In tea rows, the post-fertilization increase in CO₂ flux under HMT was 43.1%
350 lower than that under CKT, whereas SMT showed a 9.2% higher increase. In the inter-
351 row zones, HMG reduced the fertilization-induced increase by 10.4% compared to
352 CKG, while SMG amplified it by 40.1%. These findings indicate that the HM treatment
353 can effectively mitigate CO₂ emissions triggered by fertilization, while SM treatment
354 may intensify them, revealing the potential of legume-based mixed green manure to

355 reduce CO₂ emissions in tea plantations. It is worth noting that the mitigation effect in
356 the inter-row zones was weaker than in the tea rows, possibly due to differences in root
357 distribution or organic matter inputs.

358 The effects of grass planting and tea pruning on CO₂ fluxes varied by treatment
359 type and location (tea row or inter-row) (Fig. 5c-d). In the CK treatment, grass planting
360 had no significant impact on CO₂ fluxes. However, the HM treatment led to a marked
361 increase after grass planting, with inter-row fluxes rising by 1.81 mg·m⁻²·min⁻¹.
362 Similarly, the SM treatment showed significant increases in both zones, with an
363 increase of 0.90 mg·m⁻²·min⁻¹ in tea rows and an inter-row increase that was 3.8 times
364 greater. These increases can be attributed to soil disturbance during sowing.

365 After tea pruning, no significant changes in CO₂ flux were observed in the CK
366 treatment. However, both HMT and SMT significantly increased CO₂ emissions in tea
367 row, with increments of 2.74 mg·m⁻²·min⁻¹ and 2.94 mg·m⁻²·min⁻¹, respectively. In the
368 inter-row zones, only the HMG treatment exhibited a significant post-pruning increase
369 of 3.25 mg·m⁻²·min⁻¹. These increases may be attributed to pruning residues covering
370 the green manure surface, which could elevate soil temperature and moisture, thereby
371 enhancing soil respiration and CO₂ emissions.

372

373 **3.4 Effects of Environmental Factors on CO₂ Fluxes**

374 Significant differences in soil nutrient parameters were observed between tea rows
375 and inter-row zones under various green manure intercropping treatments (Fig. 6).
376 Green manure treatments generally increased soil total carbon (TC) and total nitrogen
377 (TN), with consistently higher TC and TN levels in the inter-row zones than in the tea
378 rows (Fig. 6a-b), resulting in significantly higher C/N ratios in the tea rows (Fig. 6c).
379 Soil ammonium nitrogen (NH₄⁺-N) and nitrate nitrogen (NO₃⁻-N) concentrations were
380 also significantly greater in the inter-row zones, with the highest NH₄⁺-N found in CKG
381 (71.20 mg·kg⁻¹) and the highest NO₃⁻-N in SMG (14.56 mg·kg⁻¹). All green manure
382 treatments significantly increased soil organic carbon (SOC), the average SOC contents
383 under HM and SM were 3.6% and 9.3% higher than under CK, respectively (Fig. 6f).
384 Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) showed no

385 significant differences between tea rows and inter-row zones, but both were slightly
386 elevated under green manure treatments (Fig. 6g-h). Soil pH ranged from 3.6 to 4.5,
387 with no significant differences among treatments, although green manure application
388 slightly increased soil pH (Fig. 6i).

389 During the monitoring, soil temperature ranged from 2.3–41.8°C in CK, 3.0–
390 37.6°C in HM, and 3.5–36.6°C in SM. Average soil temperatures in HM and SM were
391 4.5% and 3.9% lower than in CK, indicating a cooling effect of green manure.
392 Additionally, bulk density was reduced by 8.9% and 5.0% in HM and SM compared to
393 CK, while total porosity increased by 5.3% and 3.0%, and WFPS decreased by 29.1%
394 and 11.1%, respectively. These results suggest that green manure intercropping
395 effectively reduces soil compaction and improves soil aeration. The combined effect of
396 these factors is the key to the changes in CO₂ emissions.

397 To further clarify these relationships, we examined the correlations between CO₂
398 fluxes and environmental factors under different green manure treatments (Fig. 7). CO₂
399 fluxes across nearly all treatments were significantly positively correlated with air
400 temperature (AT) and soil temperature (ST) ($r > 0.5, p < 0.05$), suggesting temperature
401 as a major driver of CO₂ emissions in tea plantations. Treatment-specific differences
402 were also apparent: in green manure treatments (HM and SM), both TC and SOC in tea
403 rows and inter-row zones showed negative correlations with CO₂ flux, whereas
404 volumetric water content (VWC) showed significant positive correlation ($r > 0.5, p <$
405 0.05). In contrast, VWC was negatively correlated with CO₂ flux under CK treatment.
406 These findings suggest that green manure intercropping alters soil pore structure and
407 moisture regimes, thereby modifying CO₂ emission dynamics compared to bare soil
408 conditions. Additionally, environmental controls on CO₂ fluxes differed between tea
409 rows and inter-row zones. Emissions in tea rows appeared less sensitive to
410 environmental fluctuations, likely due to the moderating effects of tea canopy coverage
411 and root systems.

412 Canonical correspondence analysis (CCA) was further performed to examine the
413 effect of green manure intercropping patterns and soil properties on CO₂ emissions in
414 tea rows and inter-row zones (Fig. 8). The first two CCA axes explained 52.79% and

415 11.15% of the total variance, respectively. CCA1 was primarily associated with NH_4^+ -
416 N, NO_3^- -N, SOC, and the C/N ratio, while CCA2 was mainly linked to TN, TC, MBN,
417 MBC, and pH. These results indicate distinct environmental drivers of CO_2 emissions
418 between the two spatial zones. In tea rows, CO_2 flux was positively associated with
419 NO_3^- -N and the C/N ratio, with relatively minor influence from pH. The influencing
420 soil factors were similar for CKT and SMT, whereas HMT displayed a distinct pattern,
421 likely attributable to the presence of leguminous green manure. In inter-row zones, SOC
422 emerged as the dominant factor controlling CO_2 fluxes in HMG and SMG treatments,
423 whereas NH_4^+ -N was the key driver in CKG. Moreover, TC, TN, MBC, and MBN all
424 showed positive associations with inter-row CO_2 fluxes, with consistent soil drivers
425 under HMG and SMG that differed from CKG, indicating that green manure
426 significantly affects soil- CO_2 interactions.

427

428 **4 Discussion**

429 **4.1 CO_2 Flux Dynamics under Green Manure Intercropping**

430 This study revealed pronounced seasonal variations in CO_2 fluxes from tea
431 plantations, which were closely aligned with fluctuations in air temperature (Fig. 2a-c).
432 In spring, rising temperatures enhanced both plant and microbial respiration, leading to
433 a sharp increase in CO_2 emissions (Yan et al., 2022). During summer, when
434 temperatures reached their annual peak, intensified microbial activity accelerated the
435 decomposition of soil organic matter, resulting in the highest CO_2 fluxes of the year
436 (Allison et al., 2010). In autumn, declining temperatures and light availability reduced
437 microbial activity and soil respiration, thereby lowering CO_2 emissions (Liu et al.,
438 2020). In winter, low temperatures significantly inhibited both plant and microbial
439 respiration, causing CO_2 fluxes to drop to their annual minimum (Schnecker et al.,
440 2023). These seasonal flux patterns were consistent throughout the two-year
441 observation period, indicating the pivotal role of temperature in regulating CO_2
442 emissions in tea plantations (Chen et al., 2021).

443 Compared with the CK treatment, HM and SM increased annual cumulative CO_2
444 emissions by 3.3% and 7.9%, respectively, revealing that green manure intercropping

445 significantly elevated total CO₂ emissions. Similar findings have been reported in
446 previous studies. For example, Lee et al. (2021) observed consistently higher
447 cumulative CO₂ emissions in cropland soils under green manure treatments than under
448 fallow conditions. A meta-analysis by Muhammad et al. (2019) also showed that the
449 use of cover crops generally increases CO₂ emissions compared with bare soil. This
450 effect can be attributed to two possible mechanisms: 1) green manure crops introduce
451 exogenous carbon inputs into the soil, which stimulates CO₂ release (Ho et al., 2021);
452 and 2) green manure intercropping reduces soil bulk density and increases total porosity
453 (Song et al., 2016), thereby improving soil aeration and promoting aerobic microbial
454 activity. The enhanced microbial activity accelerates the decomposition and
455 mineralization of soil organic matter, consequently increasing CO₂ emissions (Chen et
456 al., 2019). By contrast, under the CK treatment, higher WFPS and greater soil
457 compaction may have inhibited gas diffusion and limited CO₂ release into the
458 atmosphere (Lang et al., 2017).

459 Diurnal variations of CO₂ fluxes were influenced by both seasonal dynamics and
460 the growth stages of green manure crops. CO₂ fluxes fluctuated most sharply during
461 spring and summer, with temperature identified as the primary driver of daily flux
462 patterns (Pang et al., 2019). In spring, negative CO₂ flux peaks were observed in tea
463 rows under HMT and SMT around 08:00, due to low morning temperatures suppressing
464 microbial respiration. Moreover, the presence of easily degradable organic matter from
465 green manure may have diverted microbial metabolism toward biomass accumulation
466 rather than complete mineralization to CO₂. In summer, distinct spatial differences
467 appeared between tea rows and inter-row zones. In tea rows, CO₂ fluxes under the CKT
468 treatment were significantly higher than those under HMT and SMT, while in the inter-
469 row zones, fluxes under HMG and SMG were higher than those under CKG. This
470 spatial heterogeneity highlights the dual role of green manure: in tea rows, the shading
471 effect of green manure canopy reduced soil temperatures, thereby inhibiting microbial
472 respiration; in contrast, inter-row zones were exposed to direct sunlight, root exudates
473 and decomposing plant residues provided additional carbon sources. Under favorable
474 thermal conditions, this stimulated microbial activity and thus increased CO₂ emissions

475 (Gui et al., 2024). In autumn and winter, CO₂ flux peaks were mostly recorded in the
476 afternoon, possibly due to rising temperatures reaching a threshold that accelerated
477 enzymatic reactions and microbial metabolism, enhancing root and soil respiration and
478 thus elevating CO₂ emissions (Dove et al., 2021).

479

480 **4.2 Influence of Cultivation Management on CO₂ Fluxes**

481 Fertilization, pruning, and soil tillage with cover cropping are critical
482 anthropogenic management practices in tea plantations that significantly affect CO₂
483 flux dynamics. Fertilizer application, in particular, is a major contributor to agricultural
484 greenhouse gas emissions, with emission strength influenced by the type, amount, and
485 method of application (Wang et al., 2024). In this study, the application of rapeseed
486 cake and compound fertilizers significantly increased soil CO₂ fluxes, especially within
487 tea rows. This increase can be attributed to two main factors: 1) the input of exogenous
488 organic matter enriched soil organic carbon content; and 2) trench fertilization caused
489 physical disturbance, disrupting soil aggregates and accelerating the decomposition of
490 soil organic carbon. These disturbances stimulated the abundance and metabolic
491 activity of aerobic heterotrophic microbes, promoting organic matter mineralization
492 and resulting in elevated CO₂ emissions (Chappell et al., 2015; Struck et al., 2020).
493 Intensive pruning conducted in May and August further contributed to increased CO₂
494 fluxes. Pruning substantially reduces the photosynthetic biomass of tea plants,
495 diminishing their carbon sequestration capacity. Simultaneously, the resulting litterfall
496 provides abundant substrates for microbial respiration (Pang et al., 2019). The
497 combined effect of reduced carbon uptake and increased decomposition substrates leads
498 to a rapid short-term increase in CO₂ emissions after pruning.

499 Previous studies have demonstrated that intercropping systems introduce readily
500 decomposable carbon through root exudates, while green manure decomposition
501 increases organic matter inputs and improves soil organic carbon storage (Gui et al.,
502 2024). In this study, CO₂ fluxes in inter-row zones were significantly higher than the
503 control during the wilting and decomposition stages of green manure, suggesting that
504 microbial activity was enhanced during these periods, thereby accelerating the

505 decomposition and transformation of organic matter and intensifying soil respiration.
506 Additionally, soils under green manure treatments exhibited lower annual average
507 temperatures compared to the control, indicating that intercropping with green manure
508 moderated surface soil temperatures and reduced daily temperature fluctuations. This
509 effect was particularly pronounced in summer, when green manure not only reduced
510 inter-row CO₂ emissions but also improved the microclimatic conditions of the tea
511 plantation.

512 Traditional CO₂ flux measurements in tea plantations have mostly focused only on
513 tea rows, often neglecting inter-row soil emissions (Yao et al., 2015; Chen et al., 2021).
514 In this study, static chambers were parallelly employed to measure CO₂ fluxes in tea
515 rows and inter-row areas, enabling a more accurate understanding of CO₂ emissions.
516 Results showed that inter-row CO₂ fluxes were significantly higher than those in tea
517 rows ($p < 0.05$), accounting for 52.6%, 57.3%, and 60.8% of the annual cumulative CO₂
518 emissions under CK, HM, and SM treatments, respectively. These findings emphasize
519 the substantial contribution of inter-row zones to overall CO₂ emissions. This
520 discrepancy is due to differences in management intensity: fertilization and tillage are
521 commonly performed in inter-row areas, while the soil beneath tea canopies
522 experiences minimal disturbance (Hirono and Nonaka, 2012). Moreover, pruning
523 residues often accumulate in inter-row zones, further intensifying microbial activity and
524 CO₂ emissions in these areas. The cumulative CO₂ emissions under HMG and SMG
525 treatments were significantly lower in the second year. ~~This reduction may be~~
526 ~~attributed to reduced human disturbance and the regulatory effects of green manure~~
527 ~~(Gui et al., 2024). The decrease in emissions can be attributed to the gradual attenuation~~
528 ~~of the carbon priming effect induced by soil disturbance during the initial experimental~~
529 ~~phase (Zhou, 2025), coupled with the long-term positive effects of green manure on~~
530 ~~enhancing soil physical structure and ecosystem stability (Gui et al., 2024). The~~
531 ~~increase in green manure biomass in the following year indicates that the green manure~~
532 ~~system is transitioning from an initially disturbed and unstable state toward a more~~
533 ~~productive and carbon-sequestration-enhanced stable state (Figure A1). This trend not~~
534 ~~only reflects the improved functioning of the soil ecosystem but also serves as an~~

535 important driver for further carbon sequestration, contributing significantly to the
536 reduction in inter-row CO₂ emissions observed in the following year. Therefore, long-
537 term and systematic monitoring of inter-row soil CO₂ emissions is essential for
538 accurately assessing the carbon dynamics and mitigation potential of tea plantation
539 ecosystems.

540

541 **4.3 Differences in Environmental Drivers**

542 Soil CO₂ fluxes are regulated by multiple environmental factors, including
543 photosynthetic activity or vegetation productivity (Tang et al., 2005), and soil properties
544 such as temperature and moisture (Liu et al., 2023; Widanagamage et al., 2025). Among
545 them, temperature is widely recognized as a primary driver of seasonal variation in soil
546 respiration (Lang et al., 2017). Our results showed that CO₂ fluxes in both tea rows and
547 inter-row areas were significantly correlated with soil and air temperatures under
548 different green manure treatments (Fig. 7). In addition, carbon and nitrogen
549 transformation processes driven by microorganisms are closely coupled. Nitrification
550 and denitrification alter NO₃⁻-N and NH₄⁺-N levels, thereby influencing soil
551 physicochemical properties and microbial activity. As a result, CO₂ emissions exhibit
552 significant positive correlations with nitrogen mineralization, denitrification, and N₂O
553 emissions (Dai et al., 2020). This carbon–nitrogen coupling may interact with the
554 distinctive nutrient uptake characteristics of tea plants, which are ammonium-preferring
555 species with rapid NH₄⁺ assimilation (Xin et al., 2024). In our study, CO₂ fluxes under
556 the CKG treatment were positively correlated with NH₄⁺-N content (Fig. 9). NH₄⁺-N
557 levels under this treatment (71.20 mg·kg⁻¹) were significantly higher than in the SMG
558 and HMG treatments, whereas the corresponding soil pH value (3.97) was significantly
559 lower ($p < 0.05$). This concurrent high NH₄⁺-N level and strong acidification is due to
560 ammonium accumulation under conventional fertilization and subsequent H⁺ release
561 during nitrification (Chen et al., 2021). By contrast, the soil pH under green manure
562 intercropping treatments increased by 0.02–0.11 units compared to the CK treatment
563 (Fig. 6i), suggesting that root exudates and organic matter inputs from green manure
564 buffered soil acidification by reducing H⁺ release during NH₄⁺ nitrification. Moreover,

565 the NO_3^- -N concentration in the SMG treatment (14.56 mg kg^{-1}) was significantly
566 higher than that in other treatments (Fig. 6e), due to the high biomass of *Vulpia myuros*
567 C., which may reduce nitrate losses via runoff or leaching. Its active root system also
568 improved soil aeration, inhibiting denitrification under anoxic conditions.

569 SOC content reflects the dynamic balance between organic matter inputs and
570 decomposition (Mo et al., 2024). In our study, SOC levels in the HMT and SMT
571 treatments were higher than those in the CKT treatment, while their cumulative annual
572 CO_2 emissions were lower. This indicates that increasing SOC storage can help mitigate
573 greenhouse gas emissions, consistent with findings by Han et al. (2022). However, the
574 HMG and SMG treatments exhibited much higher SOC levels than CKG, while their
575 cumulative CO_2 emissions exceeded those of CKG. This implies that once SOC
576 accumulation surpasses a certain threshold, the excess carbon supply may stimulate
577 microbial activity and subsequently increase CO_2 emissions (Lim and Choi, 2014).
578 Interestingly, recent studies reveal that SOC thresholds can modulate the impact of
579 nitrogen fertilization on carbon sequestration. In SOC-poor soils, nitrogen inputs tend
580 to promote carbon accumulation and soil aggregation, enhancing SOC storage.
581 Conversely, in SOC-rich soils, nitrogen fertilization may enhance microbial metabolic
582 efficiency and increase microbial residue production (Ling et al., 2025). Studies in
583 different climatic zones of China have revealed that SOC thresholds are influenced by
584 factors such as climate and soil type. In the maritime monsoon climate zone, dual
585 thresholds for NO_3^- -N and extractable iron (Fe) have been identified, beyond which
586 their marginal effects on SOC shift significantly. In the continental monsoon climate
587 zone, SOC content increases markedly once a critical threshold of TN is exceeded (Cui,
588 2025). Additionally, research in alpine ecosystems has shown that SOC components
589 vary along elevation gradients and exhibit distinct thresholds (Zhang, 2025). These
590 insights provide a new perspective for interpreting our results and highlight the
591 importance of identifying threshold values under multifactorial interactions to better
592 assess their effects on CO_2 emissions.

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

595 Green manure, as a fresh plant residue with a relatively low C/N ratio, can be rapidly
596 decomposed by soil microbes after incorporation, thus maintaining or enhancing SOC
597 levels (Li et al., 2024), which aligns with our observations (Fig. 7d). MBC and MBN
598 are generally considered closely linked to SOC (Gao et al., 2022). However, in our
599 study, a significant positive correlation between MBC and SOC was only observed in
600 the CKT treatment. The lack of correlation under green manure treatments may be due
601 to the rapid and excessive input of exogenous carbon, which complicates the
602 relationship between these variables. No significant differences in MBC and MBN
603 levels were found among green manure treatments, and both showed weak correlations
604 with CO₂ fluxes (Fig. 8), suggesting that MBC and MBN are not key drivers of CO₂
605 emissions in tea plantations.

606

607 **5 Conclusion**

608 This study revealed the regulation of CO₂ fluxes in tea plantations under different
609 green manure intercropping treatments. Green manure significantly influenced CO₂
610 flux dynamics, with pronounced seasonal variations, higher fluxes in summer and
611 autumn and lower fluxes in spring and winter. CO₂ emissions from inter-row areas were
612 consistently higher than those from tea rows. CO₂ fluxes in the SM and HM treatments
613 were significantly lower than in the CK treatment within tea rows, while the opposite
614 trend was observed in inter-row areas, suggesting distinct spatial responses to green
615 manure intercropping. Over the observation period, the HM and SM treatments reduced
616 CO₂ emissions from tea rows by 7.1%–7.9% compared to the CK, while increasing
617 inter-row emissions by 12.7%–28.9%. Inter-row CO₂ emissions accounted for 52.6%,
618 57.3%, and 60.8% of the annual cumulative CO₂ fluxes under the CK, HM, and SM
619 treatments, respectively. These findings highlight the importance of incorporating
620 spatial emission weighting into carbon accounting for agricultural ecosystems.

621 The observed spatial differences in CO₂ fluxes were closely related to variations
622 in SOC content. Our findings suggest the existence of a critical SOC threshold that
623 determines whether CO₂ emissions increase or decrease. Future research should focus
624 on quantifying such thresholds under multi-factor interactions to better assess their

625 impacts on greenhouse gas dynamics. ~~Continuous green manure intercropping over two~~
626 ~~years significantly mitigated CO₂ emissions from inter row areas. The HM treatment~~
627 ~~exhibited distinct diurnal CO₂ flux patterns and effectively suppressed fertilization-~~
628 ~~induced CO₂ emissions. These results demonstrate that green manure intercropping,~~
629 ~~particularly mixed legume and non-legume combinations, not only modifies the spatial~~
630 ~~pattern of CO₂ emissions in tea plantations but also provides a practical strategy for~~
631 ~~mitigating carbon losses from managed agroecosystems. Continuous green manure~~
632 ~~intercropping over two years significantly reduced inter-row CO₂ emissions, and the~~
633 ~~HM treatment suppressed fertilization-induced emission peaks. These findings~~
634 ~~demonstrate that green manure intercropping, particularly mixed legume and non-~~
635 ~~legume combinations, can effectively alter the spatial pattern of CO₂ emissions and~~
636 ~~mitigate carbon losses. Moreover, this practice provides significant co-benefits like~~
637 ~~improved soil aeration and fertility, reduced chemical fertilizers, weed suppression, and~~
638 ~~promoted tea plant growth, thereby offsetting the extra costs in labor and seeds.~~
639 ~~Therefore, green manure intercropping emerges as a practical and multifunctional~~
640 ~~strategy for reducing carbon emissions in agroecosystems. Moreover, this practice~~
641 ~~provides multiple co-benefits, such as improving soil aeration, enhancing soil fertility~~
642 ~~to reduce chemical fertilizer use, suppressing weeds, and promoting tea plant growth~~
643 ~~factors that can economically offset the additional labor and seed costs. In summary,~~
644 ~~green manure intercropping offers a viable strategy for carbon emission reduction in~~
645 ~~agricultural ecosystems.~~

646

647

648 **Data availability**

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

651

652

653 **Author contributions**

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

659

660

661 **Competing interests**

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

663

664

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896 **Tables and Figures**897 **Table 1.** Initial basic physicochemical properties of the six treatments.

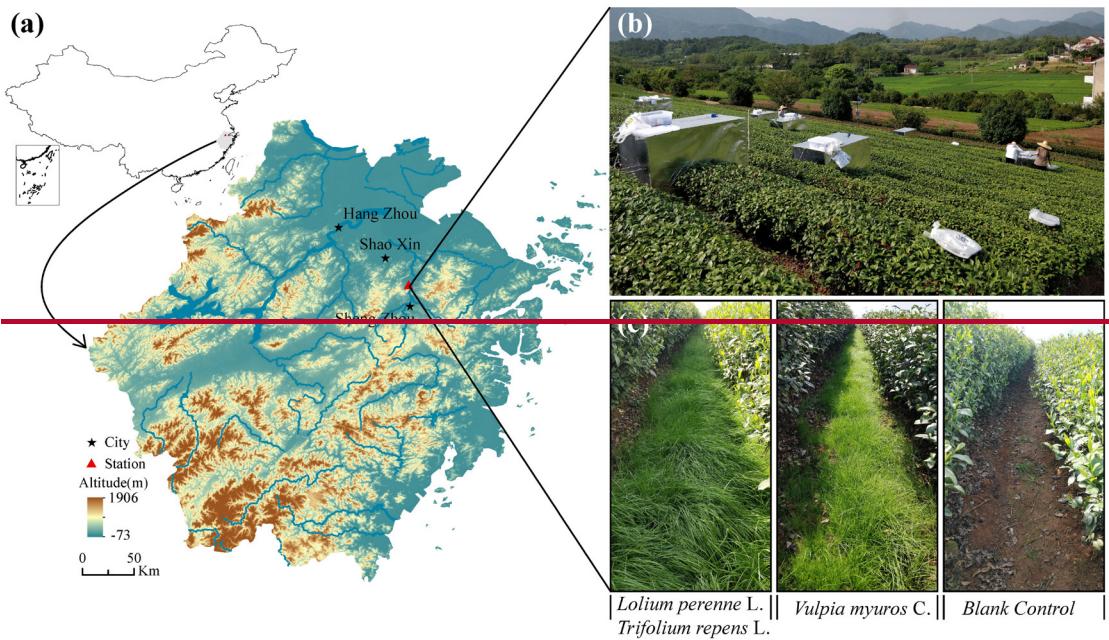
Tpye	pH	$\text{NH}_4^+ \text{-N}$ ($\text{mg} \cdot \text{kg}^{-1}$)	$\text{NO}_3^- \text{-N}$ ($\text{mg} \cdot \text{kg}^{-1}$)	TN ($\text{g} \cdot \text{kg}^{-1}$)	TC ($\text{g} \cdot \text{kg}^{-1}$)	SOC ($\text{g} \cdot \text{kg}^{-1}$)
CKT	<u>4.25±0.06^a</u>	<u>29.67±7.50^b</u>	<u>5.01±2.02^b</u>	<u>2.16±0.16^{ab}</u>	<u>22.93±2.00^a</u>	<u>25.50±2.65^a</u>
HMT	<u>4.01±0.06^{ab}</u>	<u>37.33±4.43^{ab}</u>	<u>6.32±2.25^b</u>	<u>2.01±0.09^{ab}</u>	<u>21.08±1.12^a</u>	<u>22.00±1.69^a</u>
SMT	<u>4.11±0.06^{abc}</u>	<u>30.42±8.93^b</u>	<u>5.10±1.88^b</u>	<u>1.96±0.12^b</u>	<u>20.47±1.12^a</u>	<u>21.83±1.92^a</u>
CKG	<u>4.16±0.02^{ab}</u>	<u>33.20±5.90^{ab}</u>	<u>8.59±1.46^{ab}</u>	<u>2.41±0.19^{ab}</u>	<u>25.70±1.99^a</u>	<u>21.68±1.82^a</u>
HMG	<u>3.98±0.05^b</u>	<u>37.90±7.98^{ab}</u>	<u>12.73±3.94^a</u>	<u>2.70±0.35^a</u>	<u>27.77±3.57^a</u>	<u>27.98±3.55^a</u>
SMG	<u>4.00±0.03^b</u>	<u>44.40±7.33^a</u>	<u>13.36±3.82^a</u>	<u>2.31±0.28^{ab}</u>	<u>24.92±2.86^a</u>	<u>26.88±2.53^a</u>

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

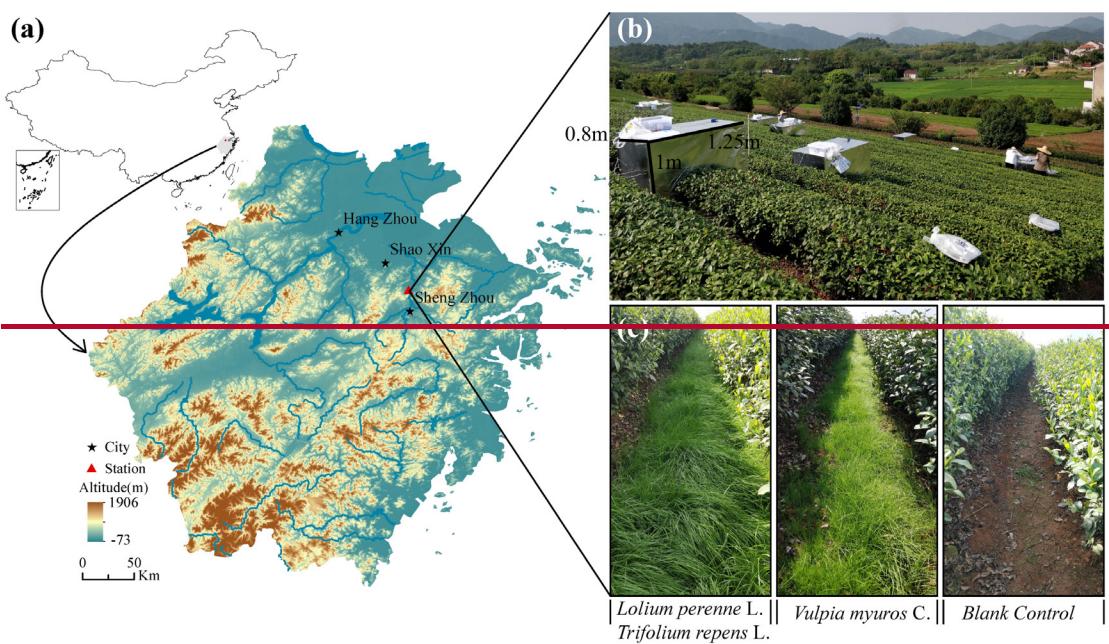
901 **Table 12.** Seasonal variation in CO₂ fluxes from tea rows and inter-row zones under
 902 different green manure intercropping treatments. **Different lowercase letters indicate**
 903 **significant differences among treatments and seasons ($P < 0.05$).**

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}

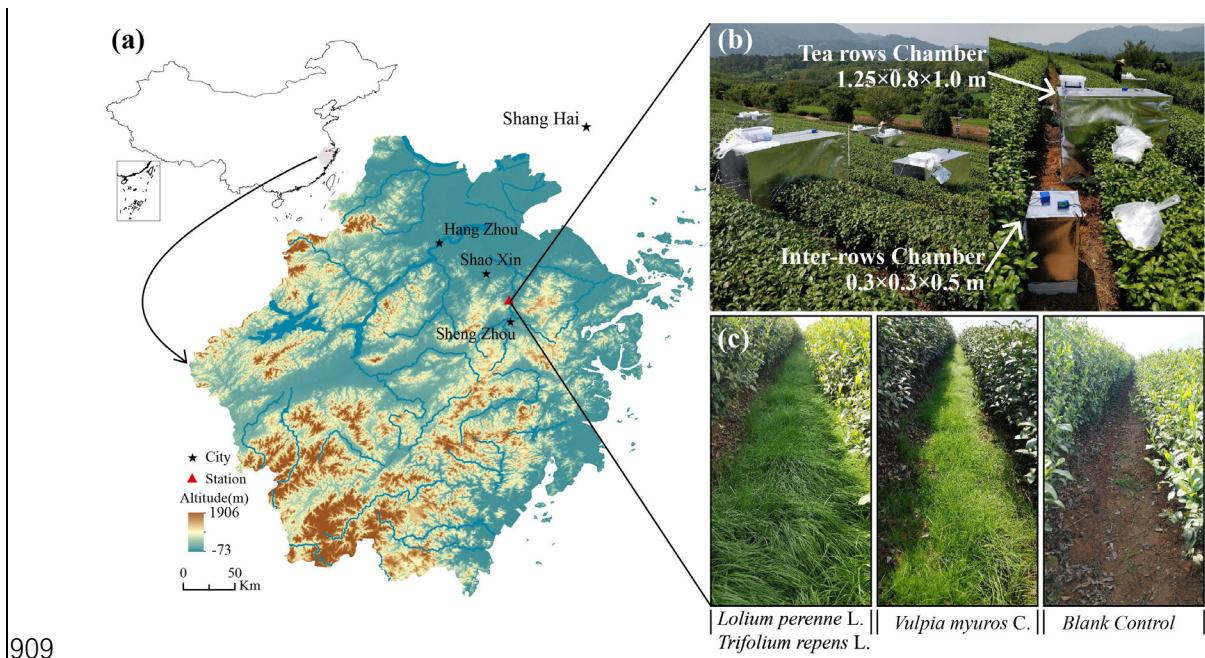
904 *Different superscript lowercase letters indicate significant differences among
 905 treatments and seasons ($p < 0.05$). Data shown are means ± SE. CK for control; SM
 906 and HM for intercropping types, T for tea row, G for inter-row.



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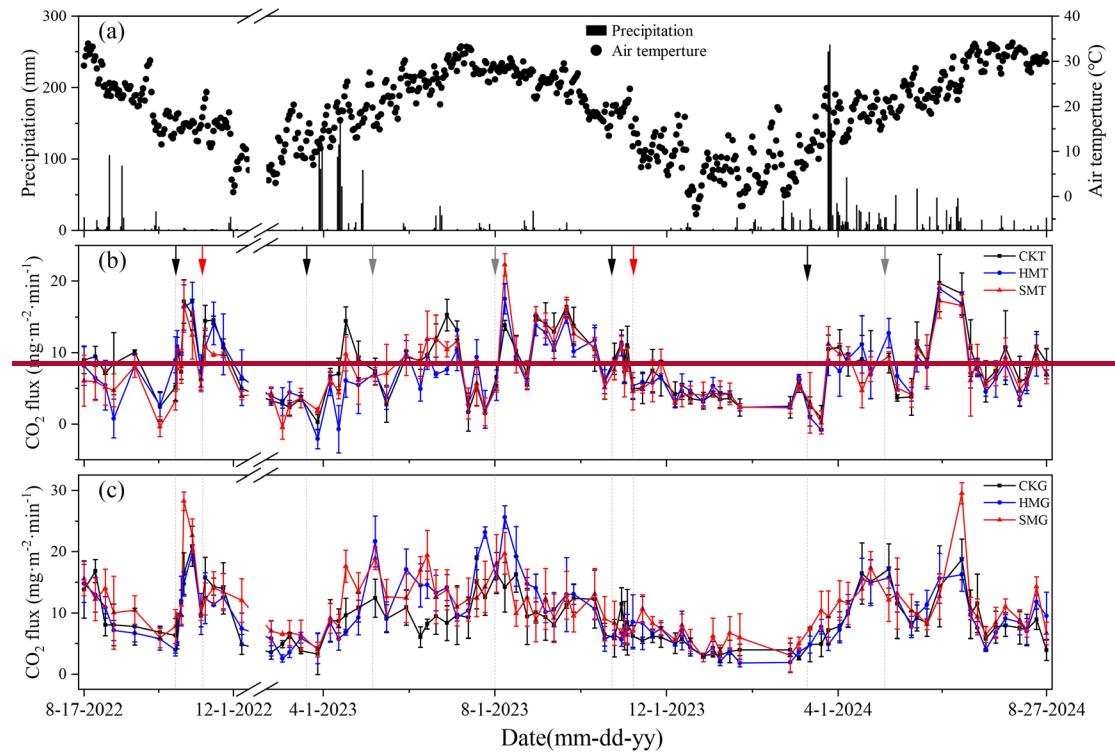
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910 **Figure 1.** (a) Geographic location of the study area in Shengzhou City, Zhejiang
 911 Province, China; (b) field layout of the tea plantation experiment; (c) photos of *Lolium*
 912 *perenne* L. and *Trifolium repens* L. plot, and *Vulpia myuros*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

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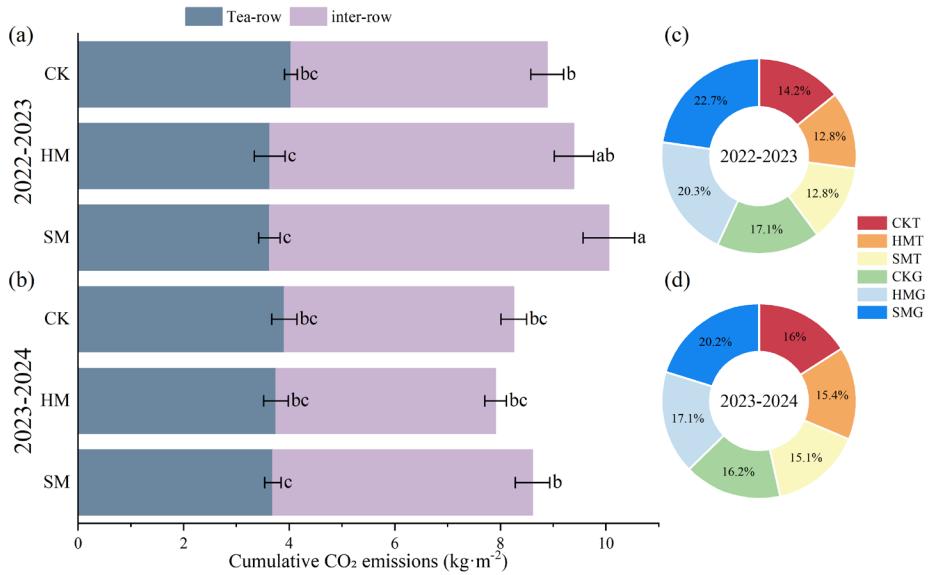


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Figure 2. Dynamics of (a) air temperature and precipitation, (b) CO₂ fluxes from tea rows, and (c) CO₂ fluxes from inter-rows during the observation period (2022–2024). Black, green and orange arrows represent the timings of fertilization, grass planting and tea pruning, respectively. Flux data are presented as mean \pm SE. CK for control; SM

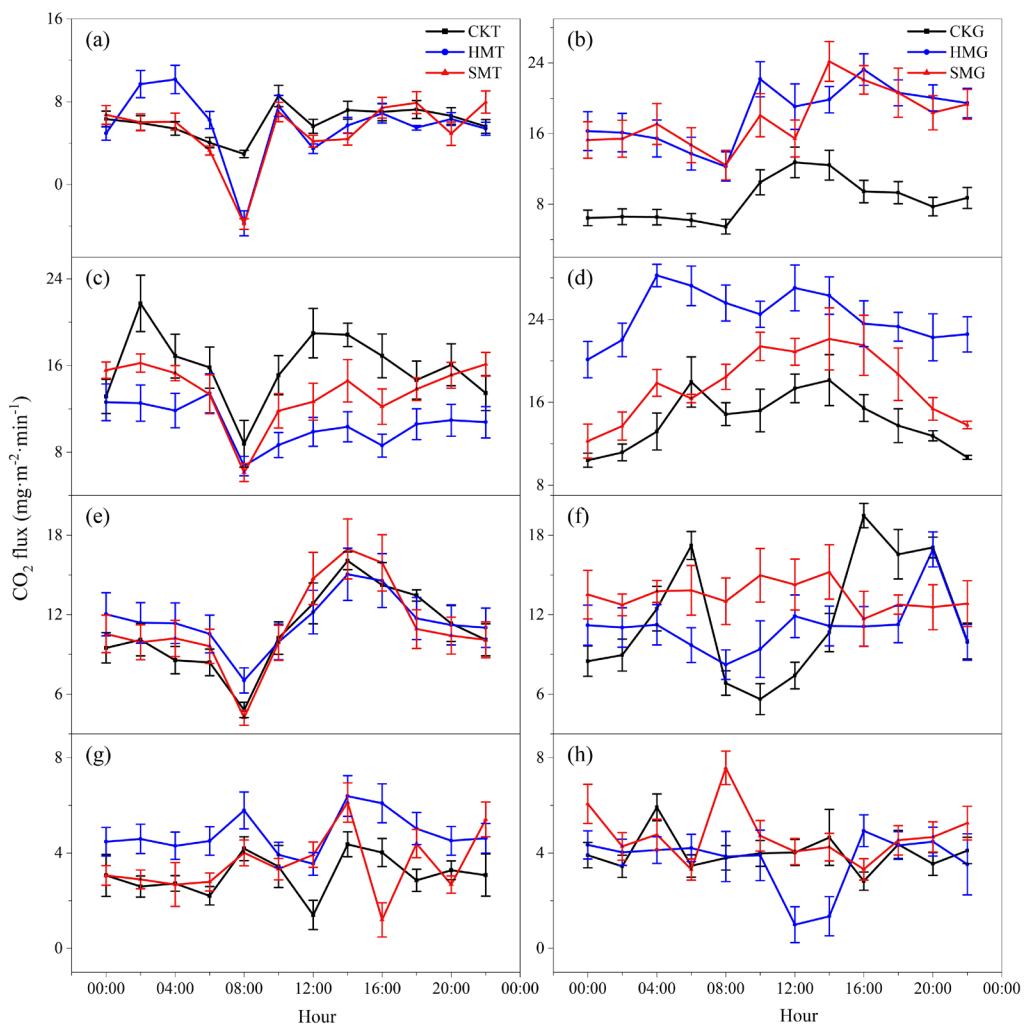
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and HM for intercropping types, T for tea row, G for inter-row.



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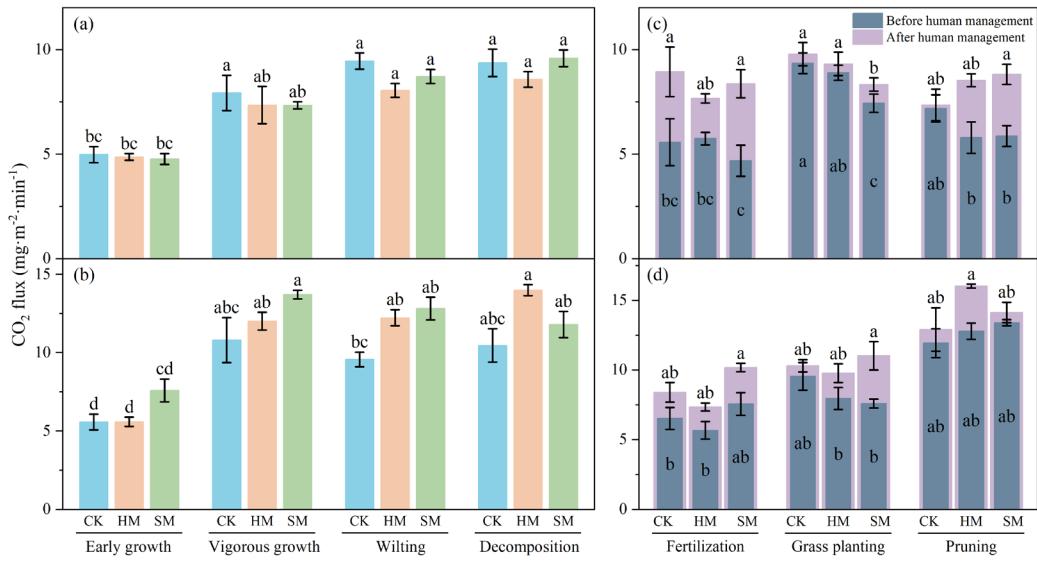
922 **Figure 3.** (a, b) Annual cumulative CO₂ emissions from tea rows and inter-rows under
923 different green manure intercropping treatments; (c, d) contribution of tea rows and
924 inter-rows to total annual CO₂ emissions under each treatment. Data shown are means
925 ± SE. Different superscript letters denote statistically significant differences ($p < 0.05$).
926 CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.



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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.

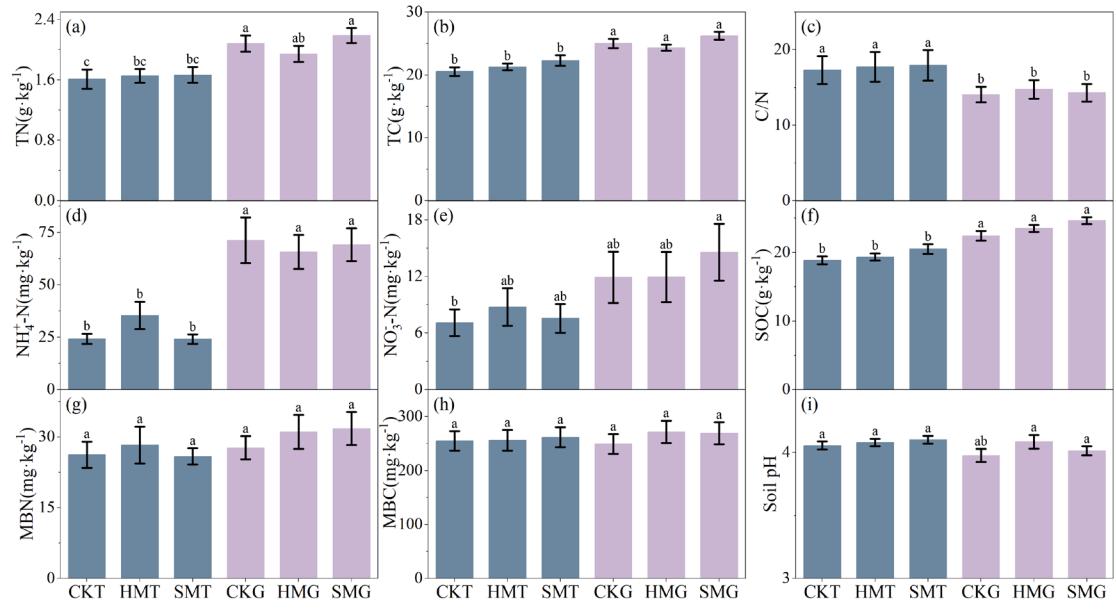


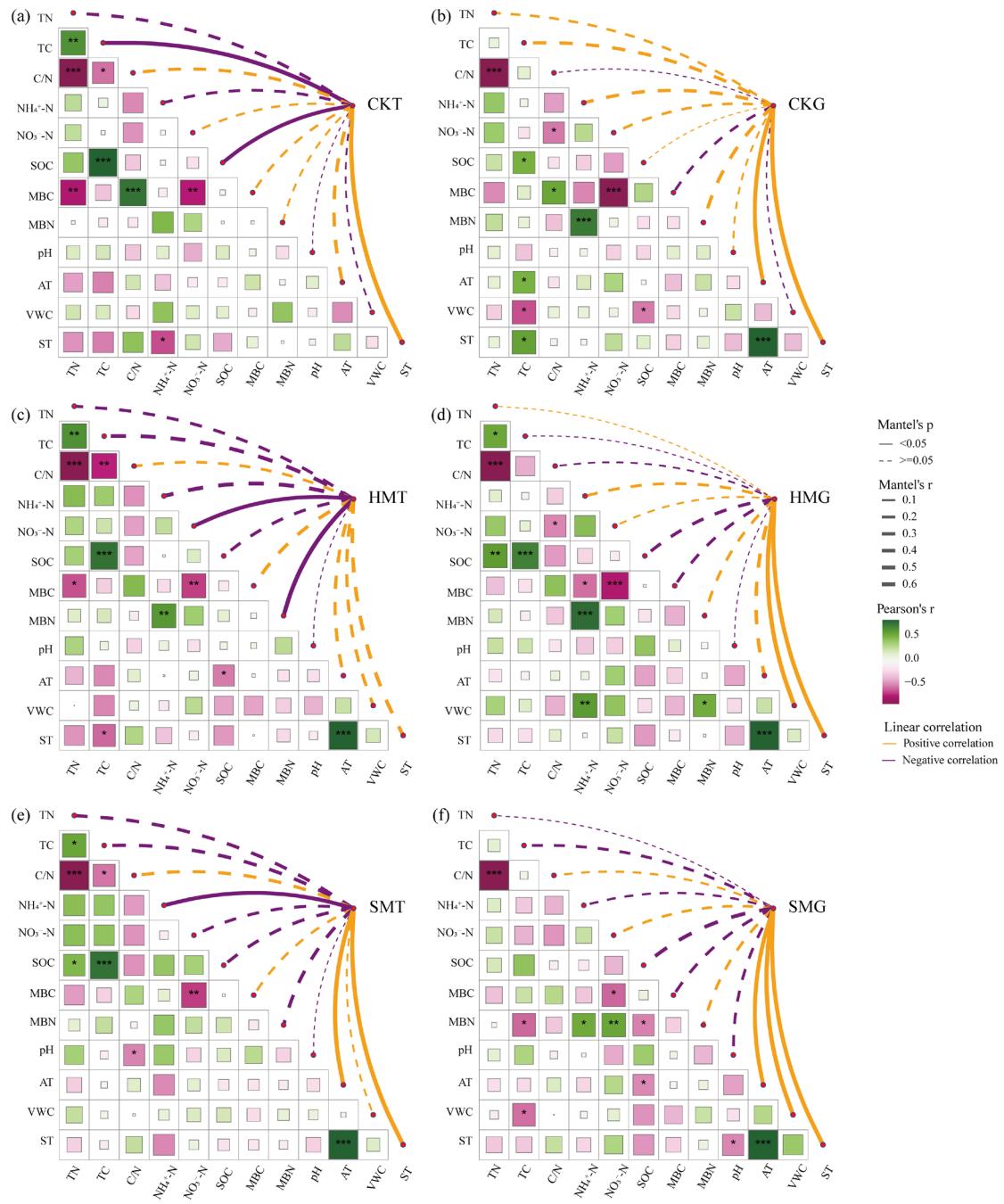
932

933 **Figure 5.** Temporal dynamics of CO₂ fluxes under green manure (a, b) growth stages
934 and (c, d) management events in tea plantations. Growth stages include: early growth
935 (mid-November to early April), vigorous growth (mid-April to late May), wilting (early
936 June to late July), and decomposition (August). CK for control; SM and HM for
937 intercropping types, T for tea row, G for inter-row.

938

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





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943

Figure 7. Pairwise correlations between environmental factors and their relationships

944

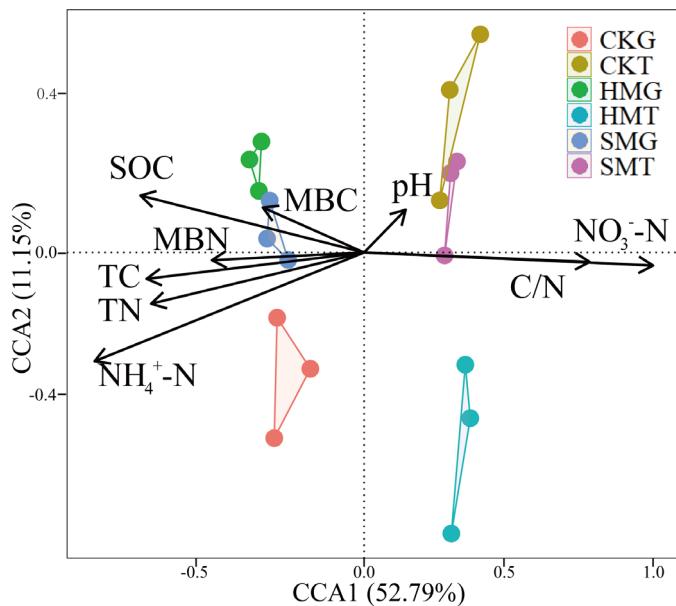
with CO₂ fluxes under different green manure treatments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

945

CK for control; SM and HM for intercropping types, T for tea row, G for inter-

946

row.



947

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

Appendix 1

The CO₂ flux was calculated using the following equation:

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

where F is the CO_2 flux ($\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$); ρ_0 is the density of CO_2 under standard conditions ($1.98 \text{ kg} \cdot \text{m}^{-3}$); P_0 and T_0 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 CO₂ concentration readings were calibrated with standard gases. Then, linear regression was performed to fit their rate of change over time. Finally, CO₂ flux was calculated using the standard flux formula by incorporating the chamber volume, base area, and the measured atmospheric pressure and temperature.

Appendix 2

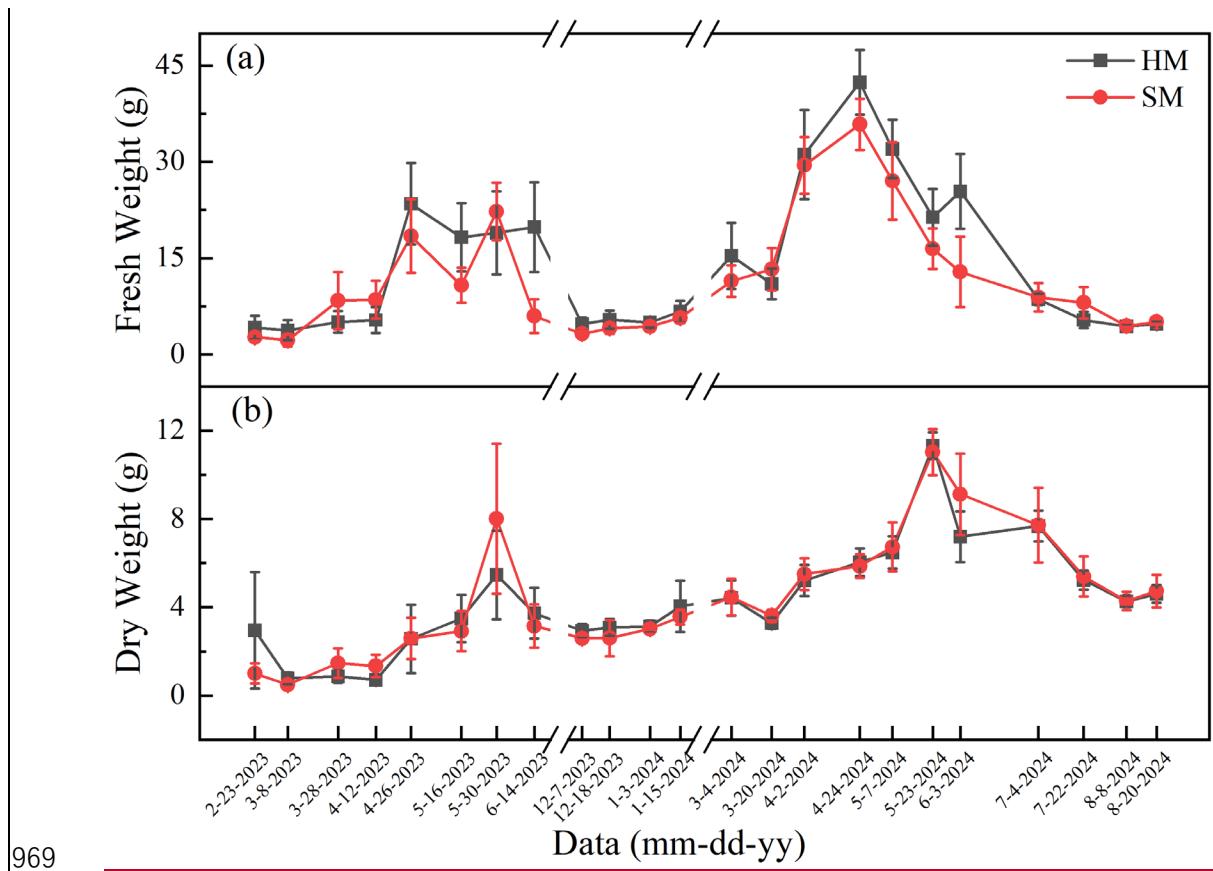


Figure A1. Temporal trend of green manure indexes

1 **Spatially Contrasting CO₂ Dynamics Driven by Green Manure**
2 **Intercropping in Subtropical Tea Plantations**

3
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19 **Abstract:**

20 Tea plantations are important contributors to greenhouse gas emissions due to intensive
21 fertilization and continuous cultivation. However, the mechanisms by which green
22 manure intercropping regulates soil CO₂ dynamics in these systems remain poorly
23 understood. We employed the static chamber method over a two-year period, with
24 sampling conducted weekly, to investigate how intercropping with *Vulpia myuros* (SM)
25 and a legume–nonlegume mixture of *Lolium perenne* and *Trifolium repens* (HM)
26 influenced spatial CO₂ flux dynamics compared with a no-intercropping control (CK)
27 from tea rows and inter-row zones in a subtropical tea plantation. Distinct seasonal
28 variations were observed, with CO₂ fluxes peaking in summer and autumn and
29 declining in spring and winter. Average tea-row fluxes were 8.7% and 9.5% lower under
30 SM and HM, respectively, compared to CK, indicating emission reductions with
31 intercropping. In contrast, average inter-row fluxes increased by 19.4% under SM and
32 7.7% under HM, demonstrating pronounced spatial contrasts. Diurnal patterns
33 generally exhibited midday peaks (12:00–14:00), especially in summer and autumn
34 across all tea-rows, and short-term CO₂ pulses were triggered by field operations such
35 as fertilization and pruning. Notably, HM effectively suppressed fertilization-induced
36 CO₂ pulses, revealing the mitigation potential of legume–nonlegume mixtures. Green
37 manure increased soil organic carbon (6.4%), lowered soil temperature (4.5%), and
38 enhanced porosity (4.2%), collectively shaping CO₂ dynamics. Multivariate analysis
39 identified soil organic carbon (SOC) and temperature as dominant flux drivers, and a
40 potential SOC threshold was detected, beyond which CO₂ emissions accelerated.
41 Compared to CK, intercropping reduced tea-row emissions by 7.1–7.9% but increased
42 inter-row emissions by 12.7–28.9% based on the two years cumulative emissions,
43 continuous intercropping significantly decreased overall inter-row emissions over time.
44 These results highlight the spatially heterogeneous nature of carbon flux regulation and
45 demonstrate the long-term potential of green manure intercropping as a climate-smart
46 management strategy in perennial agroecosystems.

47
48 **Keywords:** tea plantations, green manure, CO₂ emissions, soil factors

49 **1 Introduction**

50 Mitigating greenhouse gas (GHG) emissions to address global warming and
51 associated climate challenges remains a priority in global environmental research.
52 Among long-lived GHGs, carbon dioxide (CO₂) plays the most prominent role,
53 contributing approximately 66% to the increase in global radiative forcing (IPCC,
54 2022). In 2023, the global average atmospheric CO₂ concentration reached 420.0 ± 0.1
55 ppm, representing a 151% increase compared to pre-industrial levels (prior to 1750)
56 (WMO, 2024). Agriculture is a major emission sector, accounting for about 14% of
57 total anthropogenic CO₂ emissions (Wang et al., 2025). In China, this share is even
58 higher, with agricultural activities accounting for up to 17% of national CO₂ emissions
59 (Xu and Lin, 2017). Therefore, accurately characterizing CO₂ emission dynamics in
60 agricultural systems and scientifically informed mitigation strategies are critical for
61 advancing global GHG reduction efforts and promoting sustainable, low-carbon
62 agricultural development (Xu et al., 2024).

63 Tea (*Camellia sinensis* L.) is an important economic crop in tropical and
64 subtropical regions. Over recent decades, global tea cultivation area has expanded
65 rapidly, reaching 4.70 million hectares in 2022. China has led the most significant
66 growth, with 3.35 million hectares of tea plantations and an annual production of 2.82
67 million tons, ranking first worldwide in both area and output (FAO, 2024). To maximize
68 yield and improve tea quality, fertilizer inputs to tea cultivation area can be up to four
69 times higher than those applied to staple crops during a single growing season (Zou et
70 al., 2009; Han et al., 2013; Yao et al., 2015). In China, average annual fertilizer use in
71 tea plantations reaches 678 kg ha⁻¹, with more than 30% of plantations experiencing
72 over-application (Ni et al., 2019). Such intensive fertilization not only accelerates soil
73 acidification but also significantly increases GHGs emissions from tea plantations (Liu
74 et al., 2016; Yan et al., 2020). However, most existing studies on agricultural CO₂
75 emissions have focused on staple cropping systems such as wheat (Song et al., 2024),
76 rice (Qian et al., 2023), and maize (Zhang et al., 2020), while studies on CO₂ emissions
77 from tea plantations remain limited.

78 For instance, Lang et al. (2017) reported that intercropping rubber trees with tea

79 in the tropical forests of Xishuangbanna, China, reduced CO₂ emissions, although it
80 simultaneously weakened the soil's methane (CH₄) uptake capacity. Wanyama et al.
81 (2019) found that converting tropical montane forests into tea plantations in Africa
82 decreased annual soil CO₂ emissions to 5.6 t ha⁻¹, with emissions positively correlated
83 with soil pH and negatively correlated with the soil C/N ratio. Pang et al. (2019)
84 quantitatively assessed the net ecosystem exchange (NEE) of tea plantations in
85 southeastern China from 2014 to 2017, reporting values ranging from -182.40 to
86 -301.51 g C m⁻², indicating that tea plantations act as net carbon sinks. However, their
87 carbon sequestration potential was lower than that of other subtropical ecosystems, with
88 temperature identified as the primary factor influencing ecosystem respiration. These
89 findings suggest that CO₂ emissions from tea plantations play a non-negligible role on
90 the carbon exchange between atmosphere and tea plantations. However, the limited
91 number of studies has led to substantial uncertainty on estimating tea plantation CO₂
92 emissions, restricting our understanding of their contribution to regional and global
93 agricultural GHG budgets (Li et al., 2016; Ji et al., 2020) and hindering the development
94 of low-carbon tea plantations.

95 In response, there is a growing emphasis on the development of eco-friendly and
96 low-carbon tea plantations (Wang et al., 2022). Toward reduction of fertilizer usage and
97 higher economic efficiency, various management practices were incorporated,
98 including by using green manure. As a modernized agricultural practice, green manure
99 has been widely adopted in farming systems and serves as an important measure for
100 improving soil quality, playing a vital role in sustainable agriculture. Within the context
101 of GHGs mitigation, green manure is recognized as an effective solution for improving
102 soil quality and enhancing CO₂ sequestration in agroecosystems (Forte et al., 2017).
103 However, most studies examining the relationship between green manure and carbon
104 emissions have focused on conventional croplands such as rice and wheat.
105 Comprehensive studies have shown that appropriate green manure management can
106 significantly reduce the global warming potential (GWP) associated with fertilization
107 (Zhang et al., 2024). For instance, Gong et al. (2021) demonstrated that long-term
108 ryegrass cover in organic soybean fields effectively reduced net GWP. In contrast, other

109 studies have reported that green manure application may increase CO₂ emissions. Kim
110 et al. (2013) found that the application of Chinese milk vetch and ryegrass increased
111 winter CO₂ fluxes in paddy fields by approximately 197% and 266%, respectively.
112 Large-scale assessments have further revealed that green manure tends to increase CO₂
113 emissions, primarily due to differences in plant species and biomass inputs. Biomass
114 alone explained 63% of the variation in CO₂ emission increases, with emissions
115 declining as the C/N ratio of cover crop biomass increased. Notably, mixed sowing of
116 leguminous and non-leguminous green manures has been shown to improve residue
117 C/N ratios and reduce GHGs emissions (Muhammad et al., 2019).

118 Existing studies on GHG mitigation in tea systems have predominantly focused on
119 fertilizer reduction and substitution strategies. Wu et al. (2018) conducted a three-year
120 fertilization control experiment in southern China and found that halving nitrogen input
121 decreased nitrous oxide (N₂O) emissions by 44.5% in tea plantations. In contrast, Yao
122 et al. (2015) reported that organic fertilizer application led to a 71% increase in N₂O
123 emissions compared to conventional urea, suggesting potential trade-offs in GHG
124 outcomes. Organic amendments, such as compost or manure, have been shown to
125 improve soil fertility, enhance soil structure, porosity, and pH, and promote carbon
126 sequestration in tea plantation soils (Han et al., 2013; Wu et al., 2021). Biochar
127 application has also been identified as an effective strategy for improving soil quality
128 while simultaneously enhancing soil carbon storage and reducing emissions (Wu et al.,
129 2021). The effect of green manure intercropping on tea plantations was mainly focused
130 on improvements in tea plant growth and soil nutrient dynamics. For example,
131 intercropping with green manure species has been shown to enhance nitrogen use
132 efficiency and increase soil microbial diversity (Huang et al., 2023). The potential role
133 of green manure intercropping in mitigating GHGs emissions in tea ecosystems remains
134 poorly understood, and its interactions with key environmental factors have not been
135 fully clarified (Zhu et al., 2018). Green manure may influence CO₂ emissions by
136 altering carbon input levels and inducing soil disturbances, but the specific emission
137 characteristics and driving factors require further investigation.

138 To address these gaps, this study selected cultivated tea plantations region located

139 in the east of China, where is recognized as a very important tea cultivation area famous
140 by the tea name of Longjing. Commonly used green manure species in tea systems
141 (*Vulpia myuros* C., *Lolium perenne* L., and *Trifolium repens* L.) were selected for
142 intercropping, covering both leguminous and non-leguminous species, under
143 monoculture and mixed-sowing configurations. CO₂ flux were carried out in both tea
144 rows and inter-row zones across different intercropping treatments. Dynamics and key
145 influencing factors of CO₂ emissions under various green manure intercropping models
146 were analyzed, aiming to reveal the potential reasons by which green manure
147 intercropping regulates carbon fluxes in tea plantations and provide support for the
148 development of low-carbon tea plantations and sustainable regional agriculture.

149

150 **2 Methodology**

151 **2.1 Monitoring Site**

152 This study was conducted at the Comprehensive Experimental Tea Plantation Base
153 of the Tea Research Institute, Chinese Academy of Agricultural Sciences, located in
154 Shengzhou, Zhejiang Province, China (120°83'E, 29°75'N; elevation 30 m a.s.l.) (Fig.
155 1a). The site is situated in a low mountainous and hilly region of southeastern China
156 and is characterized by a subtropical monsoon climate. During the experimental period
157 (August 2022 to August 2024), the average annual temperature was approximately
158 16 °C, with an average annual precipitation of about 1400 mm. The region experiences
159 a concentrated rainy season from April to June and has a frost-free period of around
160 240 days. The tea cultivated at the site is *Jinmudan*, an elite cultivar derived from the
161 hybridization of *Tieguanyin* and *Huangdan*, and is widely planted across China. The
162 tea plantation was established in 2015; the tea plants are 8–10 years old and arranged
163 in a single-row planting pattern with a row spacing of 150 cm and a plant spacing of 40
164 cm. The soil type of the tea plantation is classified as red soil (Ultisol), a typical soil
165 type in this region, and the initial basic physicochemical properties of the six treatment
166 soils are shown in Table 1.

167

168 **2.2 Experimental Setup**

169 Three green manure intercropping treatments were established in this study: *Vulpia*
170 *myuros* C. (SM), a mixture of *Lolium perenne* L. and *Trifolium repens* L. (HM), and a
171 control treatment without intercropping (CK). The experimental tea plantation covered
172 an area of approximately 1000 m². For each treatment, three representative tea rows
173 were selected as three replicates. Adjacent treatments were separated by two tea rows
174 (~3 m), and replicate areas within the same treatment were spaced approximately 5 m
175 apart. Gas fluxes were measured in both tea rows (T) and inter-row zones (G), resulting
176 in six treatments: SMT, SMG, HMT, HMG, CKT, and CKG, with a total of 18
177 representative sampling points (Fig. 1b, c). Tea plantation followed standard
178 management practices, including fertilization, pruning, and tillage. Basal fertilization
179 and tillage were conducted in middle of October, followed by green manure sowing in
180 early November. Urea was applied as a topdressing in early February. Tea leaves were
181 harvested in end of March, and pruning was normally conducted in May and July.

182 Gas sampling was performed using the static chamber–gas chromatography
183 method. The dimensions of the static chambers were 1.25 × 0.8 × 1.0 m for tea rows and
184 0.3 × 0.3 × 0.5 m for inter-row zones (Fig. 1b). Each chamber was equipped with an
185 internal fan to ensure uniform gas mixing. To avoid rapid heating due to sunlight, the
186 chambers were wrapped with aluminum foil and sponge, functioning as dark chambers.
187 To minimize disturbance, chamber bases with water grooves were installed one month
188 in advance at each sampling point, inserted 15 cm into the soil. During sampling, water
189 was added to the grooves, and the chamber was securely sealed onto the base to create
190 a closed environment. Four gas samples were collected at 7-minute intervals using gas
191 sampling bags.

192 For seasonal monitoring, sampling was conducted once per week between 09:00
193 and 11:00 a.m. (local time). Intensive sampling was also carried out following key
194 management events such as fertilization and pruning. Diurnal variation was monitored
195 over three consecutive days in January, April, July, and October, representing winter,
196 spring, summer, and autumn, respectively. During these campaigns, gas samples were
197 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). CO₂ 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 CO₂ 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 (R²) 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 (NO₃⁻-N) and ammonium nitrogen (NH₄⁺-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.

227 **2.3 Data Processing**

228 The flux refers to the amount of gas exchanged per unit time and unit area. A
229 positive value indicates net emission to the atmosphere, while a negative value indicates
230 net uptake from the atmosphere (Yao et al., 2015; Zhang et al., 2020). Based on the flux
231 measurements, cumulative CO₂ emissions under different green manure intercropping
232 treatments were also estimated. All data analyses and visualizations were performed
233 using R software. Two-way analysis of variance (Two-way ANOVA) was employed to
234 assess the effects of treatment type and observation period on CO₂ fluxes and soil
235 physicochemical properties. Spearman correlation analysis and Mantel tests were used
236 to examine the relationships between CO₂ flux and environmental variables under
237 different green manure intercropping treatments. Canonical correspondence analysis
238 (CCA) was applied to comprehensively evaluate the influence of soil physicochemical
239 properties on CO₂ emissions. Data shown are means \pm standard error (SE). In all
240 statistical tests, the level of significant differences and correlations was set at $p < 0.05$.

241

242 **3 Results**

243 **3.1 Long-term Variation of CO₂ Fluxes under Green Manure Intercropping**

244 Figure 2 illustrates the long-term trends of key environmental variables and CO₂
245 fluxes in the tea plantation throughout the observation period. Overall, CO₂ fluxes from
246 both tea-row and inter-row zones displayed distinct seasonal patterns: higher in summer
247 and autumn, and lower in spring and winter. The seasonal differences between the warm
248 (summer and autumn) and cool (spring and winter) periods were statistically significant
249 (Table 2). The temporal dynamics of CO₂ fluxes closely tracked the trends in air
250 temperature (Fig. 2a), suggesting that temperature is a key driver of soil respiration in
251 tea plantations. Annual fluctuations in CO₂ fluxes were also strongly influenced by field
252 management activities. For example, a sharp increase in CO₂ emissions was observed
253 following basal fertilization in October, and another rise occurred in March of the
254 following year after topdressing and with the onset of warmer temperatures, ultimately
255 peaking in summer (Fig. 2b-c). The effects of management activities are further detailed
256 in Section 3.3.

257 In tea rows, the annual mean CO_2 fluxes under HMT and SMT treatments were
258 $7.35 \pm 0.44 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ and $7.41 \pm 0.45 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$, respectively, both lower than
259 that of the control (CKT: $8.12 \pm 0.46 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) (Fig. 2b). In contrast, in inter-row
260 zones, the annual mean CO_2 fluxes were significantly higher under HMG (9.77 ± 0.54
261 $\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) and SMG ($10.83 \pm 0.52 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) compared to the control (CKG:
262 $9.07 \pm 0.44 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) ($p < 0.05$) (Fig. 2c). Across seasons, CKT generally
263 exhibited higher CO_2 fluxes than HMT and SMT, except during winter. In the inter-row
264 zones, both HMG and SMG showed significantly higher fluxes than CKG in summer,
265 while SMG consistently had significantly higher CO_2 emissions than both CKG and
266 HMG during the remaining seasons ($p < 0.05$) (Table 2).

267 Overall, green manure intercropping significantly increased CO_2 emissions from
268 inter-rows, but reduced emissions in tea rows. In terms of cumulative annual emissions,
269 HMT and SMT resulted in $3.69 \text{ kg} \cdot \text{m}^{-2}$ and $3.66 \text{ kg} \cdot \text{m}^{-2}$ of CO_2 emissions, respectively,
270 both lower than the $3.97 \text{ kg} \cdot \text{m}^{-2}$ under CKT (Fig. 3). Similarly, cumulative CO_2
271 emissions under HMG and SMG remained consistently higher than under CKG, but
272 they declined from $5.76 \text{ kg} \cdot \text{m}^{-2}$ and $6.43 \text{ kg} \cdot \text{m}^{-2}$ in the first year to $4.16 \text{ kg} \cdot \text{m}^{-2}$ and 4.92
273 $\text{kg} \cdot \text{m}^{-2}$ in the second year, respectively (Fig. 3). Two consecutive years of green manure
274 intercropping led to a gradual reduction in CO_2 emissions from inter-rows, indicating
275 its potential role in long-term emission mitigation in tea plantations. CO_2 emissions
276 from inter-rows were substantially higher than those from tea rows. Compared with the
277 control, HM and SM intercropping increased inter-row cumulative CO_2 emissions by
278 12.7% and 28.9%, respectively, while reducing tea-row emissions by 7.1% and 7.9%
279 (Fig. 3a-b). Inter-row zones accounted for 52.6%, 57.3%, and 60.8% of the total annual
280 CO_2 emissions in the CK, HM, and SM treatments, respectively (Fig. 3c-d), indicating
281 that the inter-row emissions cannot be ignored.

282

283 **3.2 Diurnal CO_2 Variations**

284 CO_2 fluxes in the tea plantation exhibited pronounced diurnal variations across all
285 seasons, particularly in spring and summer (Fig. 4), likely influenced by the growth
286 stages of green manure species. In spring, CO_2 fluxes in tea rows under all treatments

287 showed a similar diurnal trend: an initial decline followed by a rapid increase. HMT
288 and SMT reached their minimum fluxes at 08:00 (local time), with values of -3.74
289 $\text{mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ and $-3.80 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$, respectively, then rose sharply and stabilized in
290 the afternoon. The diurnal amplitudes under HMT and SMT were notably greater than
291 that of the control (CKT) (Fig. 4a). In the inter-row zones, the diurnal patterns under
292 green manure treatments differed notably from the control (Fig. 4b). CKG displayed a
293 unimodal pattern with a peak at 12:00 ($12.74 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$) and a trough at 08:00 (5.45
294 $\text{mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$), resulting in an amplitude of $7.29 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$. In contrast, HMG and
295 SMG exhibited later peaks at 16:00 ($23.26 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$) and 14:00 (24.17
296 $\text{mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$), respectively, with troughs also at 08:00 (HMG: $12.28 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$;
297 SMG: $12.43 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$). Both treatments showed substantially higher amplitudes
298 than CKG.

299 Summer exhibited the most pronounced diurnal variation of CO_2 fluxes across all
300 seasons. In tea rows, CKT, HMT, and SMT followed a bimodal pattern, with peaks at
301 02:00 and 12:00, and a trough at 08:00. Their respective diurnal amplitudes were 12.96,
302 6.70, and $10.10 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ (Fig. 4c). In the inter-rows, the amplitudes were
303 relatively lower, 7.72, 8.12, and $7.79 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ for CKG, HMG, and SMG,
304 respectively, indicating smaller fluctuations compared to tea rows (Fig. 4d). Notably,
305 summer also showed the most distinct contrast between tea rows and inter-rows: CKT
306 recorded the highest average flux in the tea rows, while CKG had the lowest in the inter-
307 rows.

308 In autumn, tea-row fluxes under all treatments exhibited a unimodal pattern, with
309 minima at 08:00 and peaks at 14:00. The diurnal amplitudes were 11.27, 8.02, and 12.75
310 $\text{mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ for CKT, HMT, and SMT, respectively (Fig. 4e). In the inter-rows, HMG
311 and SMG displayed relatively stable diurnal trends, whereas CKG showed a bimodal
312 pattern with peaks at 06:00 and 16:00, and a greater amplitude than both HMG and
313 SMG (Fig. 4f).

314 In winter, CO_2 fluxes showed the most stable diurnal variation of the year. In tea
315 rows, amplitudes were only 2.96, 2.84, and $4.92 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ for CKT, HMT, and
316 SMT, respectively (Fig. 4g). Unlike other seasons, 08:00 no longer corresponded to the

317 daily minimum but rather to a relative maximum, with daily peaks generally occurring
318 at 14:00. In inter-rows, diurnal patterns were less defined. SMG exhibited the highest
319 flux at 08:00 ($7.57 \text{ mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$), while HMG showed the lowest at 14:00 (0.99
320 $\text{mg}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$) (Fig. 4h).

321

322 **3.3 Effect of Human Management on CO₂ Fluxes**

323 CO₂ fluxes from the tea field varied significantly across different growth stages of
324 green manure, exhibiting a general increasing trend from the early growth stage to the
325 vigorous, wilting, and decomposition stages (Fig. 5a). In the tea rows, the lowest fluxes
326 were observed during the early growth stage, while the highest occurred during the
327 decomposition stage. Differences among the three treatments (CKT, HMT, and SMT)
328 were minimal during the early growth but became more apparent in the subsequent
329 stages. Notably, during the vigorous stage, both HMT and SMT treatments reduced CO₂
330 emissions compared to CKT. In contrast, the impact of green manure growth on CO₂
331 fluxes was more pronounced in the inter-row zones (Fig. 5b). At all growth stages, CO₂
332 fluxes under the HMG and SMG treatments were significantly higher than those under
333 CKG, with the largest differences observed during the wilting stage. Peak emissions
334 occurred during the decomposition stage for HMG and during the wilting stage for
335 SMG.

336 Fertilization substantially increased CO₂ emissions across the tea plantation (Fig.
337 5c-d). In tea rows, the post-fertilization increase in CO₂ flux under HMT was 43.1%
338 lower than that under CKT, whereas SMT showed a 9.2% higher increase. In the inter-
339 row zones, HMG reduced the fertilization-induced increase by 10.4% compared to
340 CKG, while SMG amplified it by 40.1%. These findings indicate that the HM treatment
341 can effectively mitigate CO₂ emissions triggered by fertilization, while SM treatment
342 may intensify them, revealing the potential of legume-based mixed green manure to
343 reduce CO₂ emissions in tea plantations. It is worth noting that the mitigation effect in
344 the inter-row zones was weaker than in the tea rows, possibly due to differences in root
345 distribution or organic matter inputs.

346 The effects of grass planting and tea pruning on CO₂ fluxes varied by treatment

347 type and location (tea row or inter-row) (Fig. 5c-d). In the CK treatment, grass planting
348 had no significant impact on CO₂ fluxes. However, the HM treatment led to a marked
349 increase after grass planting, with inter-row fluxes rising by 1.81 mg·m⁻²·min⁻¹.
350 Similarly, the SM treatment showed significant increases in both zones, with an
351 increase of 0.90 mg·m⁻²·min⁻¹ in tea rows and an inter-row increase that was 3.8 times
352 greater. These increases can be attributed to soil disturbance during sowing.

353 After tea pruning, no significant changes in CO₂ flux were observed in the CK
354 treatment. However, both HMT and SMT significantly increased CO₂ emissions in tea
355 row, with increments of 2.74 mg·m⁻²·min⁻¹ and 2.94 mg·m⁻²·min⁻¹, respectively. In the
356 inter-row zones, only the HMG treatment exhibited a significant post-pruning increase
357 of 3.25 mg·m⁻²·min⁻¹. These increases may be attributed to pruning residues covering
358 the green manure surface, which could elevate soil temperature and moisture, thereby
359 enhancing soil respiration and CO₂ emissions.

360

361 **3.4 Effects of Environmental Factors on CO₂ Fluxes**

362 Significant differences in soil nutrient parameters were observed between tea rows
363 and inter-row zones under various green manure intercropping treatments (Fig. 6).
364 Green manure treatments generally increased soil total carbon (TC) and total nitrogen
365 (TN), with consistently higher TC and TN levels in the inter-row zones than in the tea
366 rows (Fig. 6a-b), resulting in significantly higher C/N ratios in the tea rows (Fig. 6c).
367 Soil ammonium nitrogen (NH₄⁺-N) and nitrate nitrogen (NO₃⁻-N) concentrations were
368 also significantly greater in the inter-row zones, with the highest NH₄⁺-N found in CKG
369 (71.20 mg·kg⁻¹) and the highest NO₃⁻-N in SMG (14.56 mg·kg⁻¹). All green manure
370 treatments significantly increased soil organic carbon (SOC), the average SOC contents
371 under HM and SM were 3.6% and 9.3% higher than under CK, respectively (Fig. 6f).
372 Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) showed no
373 significant differences between tea rows and inter-row zones, but both were slightly
374 elevated under green manure treatments (Fig. 6g-h). Soil pH ranged from 3.6 to 4.5,
375 with no significant differences among treatments, although green manure application
376 slightly increased soil pH (Fig. 6i).

377 During the monitoring, soil temperature ranged from 2.3–41.8°C in CK, 3.0–
378 37.6°C in HM, and 3.5–36.6°C in SM. Average soil temperatures in HM and SM were
379 4.5% and 3.9% lower than in CK, indicating a cooling effect of green manure.
380 Additionally, bulk density was reduced by 8.9% and 5.0% in HM and SM compared to
381 CK, while total porosity increased by 5.3% and 3.0%, and WFPS decreased by 29.1%
382 and 11.1%, respectively. These results suggest that green manure intercropping
383 effectively reduces soil compaction and improves soil aeration. The combined effect of
384 these factors is the key to the changes in CO₂ emissions.

385 To further clarify these relationships, we examined the correlations between CO₂
386 fluxes and environmental factors under different green manure treatments (Fig. 7). CO₂
387 fluxes across nearly all treatments were significantly positively correlated with air
388 temperature (AT) and soil temperature (ST) ($r > 0.5, p < 0.05$), suggesting temperature
389 as a major driver of CO₂ emissions in tea plantations. Treatment-specific differences
390 were also apparent: in green manure treatments (HM and SM), both TC and SOC in tea
391 rows and inter-row zones showed negative correlations with CO₂ flux, whereas
392 volumetric water content (VWC) showed significant positive correlation ($r > 0.5, p <$
393 0.05). In contrast, VWC was negatively correlated with CO₂ flux under CK treatment.
394 These findings suggest that green manure intercropping alters soil pore structure and
395 moisture regimes, thereby modifying CO₂ emission dynamics compared to bare soil
396 conditions. Additionally, environmental controls on CO₂ fluxes differed between tea
397 rows and inter-row zones. Emissions in tea rows appeared less sensitive to
398 environmental fluctuations, likely due to the moderating effects of tea canopy coverage
399 and root systems.

400 Canonical correspondence analysis (CCA) was further performed to examine the
401 effect of green manure intercropping patterns and soil properties on CO₂ emissions in
402 tea rows and inter-row zones (Fig. 8). The first two CCA axes explained 52.79% and
403 11.15% of the total variance, respectively. CCA1 was primarily associated with NH₄⁺
404 N, NO₃[−]-N, SOC, and the C/N ratio, while CCA2 was mainly linked to TN, TC, MBN,
405 MBC, and pH. These results indicate distinct environmental drivers of CO₂ emissions
406 between the two spatial zones. In tea rows, CO₂ flux was positively associated with

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

415

416 **4 Discussion**

417 **4.1 CO_2 Flux Dynamics under Green Manure Intercropping**

418 This study revealed pronounced seasonal variations in CO_2 fluxes from tea
419 plantations, which were closely aligned with fluctuations in air temperature (Fig. 2a-c).
420 In spring, rising temperatures enhanced both plant and microbial respiration, leading to
421 a sharp increase in CO_2 emissions (Yan et al., 2022). During summer, when
422 temperatures reached their annual peak, intensified microbial activity accelerated the
423 decomposition of soil organic matter, resulting in the highest CO_2 fluxes of the year
424 (Allison et al., 2010). In autumn, declining temperatures and light availability reduced
425 microbial activity and soil respiration, thereby lowering CO_2 emissions (Liu et al.,
426 2020). In winter, low temperatures significantly inhibited both plant and microbial
427 respiration, causing CO_2 fluxes to drop to their annual minimum (Schnecker et al.,
428 2023). These seasonal flux patterns were consistent throughout the two-year
429 observation period, indicating the pivotal role of temperature in regulating CO_2
430 emissions in tea plantations (Chen et al., 2021).

431 Compared with the CK treatment, HM and SM increased annual cumulative CO_2
432 emissions by 3.3% and 7.9%, respectively, revealing that green manure intercropping
433 significantly elevated total CO_2 emissions. Similar findings have been reported in
434 previous studies. For example, Lee et al. (2021) observed consistently higher
435 cumulative CO_2 emissions in cropland soils under green manure treatments than under
436 fallow conditions. A meta-analysis by Muhammad et al. (2019) also showed that the

437 use of cover crops generally increases CO₂ emissions compared with bare soil. This
438 effect can be attributed to two possible mechanisms: 1) green manure crops introduce
439 exogenous carbon inputs into the soil, which stimulates CO₂ release (Ho et al., 2021);
440 and 2) green manure intercropping reduces soil bulk density and increases total porosity
441 (Song et al., 2016), thereby improving soil aeration and promoting aerobic microbial
442 activity. The enhanced microbial activity accelerates the decomposition and
443 mineralization of soil organic matter, consequently increasing CO₂ emissions (Chen et
444 al., 2019). By contrast, under the CK treatment, higher WFPS and greater soil
445 compaction may have inhibited gas diffusion and limited CO₂ release into the
446 atmosphere (Lang et al., 2017).

447 Diurnal variations of CO₂ fluxes were influenced by both seasonal dynamics and
448 the growth stages of green manure crops. CO₂ fluxes fluctuated most sharply during
449 spring and summer, with temperature identified as the primary driver of daily flux
450 patterns (Pang et al., 2019). In spring, negative CO₂ flux peaks were observed in tea
451 rows under HMT and SMT around 08:00, due to low morning temperatures suppressing
452 microbial respiration. Moreover, the presence of easily degradable organic matter from
453 green manure may have diverted microbial metabolism toward biomass accumulation
454 rather than complete mineralization to CO₂. In summer, distinct spatial differences
455 appeared between tea rows and inter-row zones. In tea rows, CO₂ fluxes under the CKT
456 treatment were significantly higher than those under HMT and SMT, while in the inter-
457 row zones, fluxes under HMG and SMG were higher than those under CKG. This
458 spatial heterogeneity highlights the dual role of green manure: in tea rows, the shading
459 effect of green manure canopy reduced soil temperatures, thereby inhibiting microbial
460 respiration; in contrast, inter-row zones were exposed to direct sunlight, root exudates
461 and decomposing plant residues provided additional carbon sources. Under favorable
462 thermal conditions, this stimulated microbial activity and thus increased CO₂ emissions
463 (Gui et al., 2024). In autumn and winter, CO₂ flux peaks were mostly recorded in the
464 afternoon, possibly due to rising temperatures reaching a threshold that accelerated
465 enzymatic reactions and microbial metabolism, enhancing root and soil respiration and
466 thus elevating CO₂ emissions (Dove et al., 2021).

467

468 **4.2 Influence of Cultivation Management on CO₂ Fluxes**

469 Fertilization, pruning, and soil tillage with cover cropping are critical
470 anthropogenic management practices in tea plantations that significantly affect CO₂
471 flux dynamics. Fertilizer application, in particular, is a major contributor to agricultural
472 greenhouse gas emissions, with emission strength influenced by the type, amount, and
473 method of application (Wang et al., 2024). In this study, the application of rapeseed
474 cake and compound fertilizers significantly increased soil CO₂ fluxes, especially within
475 tea rows. This increase can be attributed to two main factors: 1) the input of exogenous
476 organic matter enriched soil organic carbon content; and 2) trench fertilization caused
477 physical disturbance, disrupting soil aggregates and accelerating the decomposition of
478 soil organic carbon. These disturbances stimulated the abundance and metabolic
479 activity of aerobic heterotrophic microbes, promoting organic matter mineralization
480 and resulting in elevated CO₂ emissions (Chappell et al., 2015; Struck et al., 2020).
481 Intensive pruning conducted in May and August further contributed to increased CO₂
482 fluxes. Pruning substantially reduces the photosynthetic biomass of tea plants,
483 diminishing their carbon sequestration capacity. Simultaneously, the resulting litterfall
484 provides abundant substrates for microbial respiration (Pang et al., 2019). The
485 combined effect of reduced carbon uptake and increased decomposition substrates leads
486 to a rapid short-term increase in CO₂ emissions after pruning.

487 Previous studies have demonstrated that intercropping systems introduce readily
488 decomposable carbon through root exudates, while green manure decomposition
489 increases organic matter inputs and improves soil organic carbon storage (Gui et al.,
490 2024). In this study, CO₂ fluxes in inter-row zones were significantly higher than the
491 control during the wilting and decomposition stages of green manure, suggesting that
492 microbial activity was enhanced during these periods, thereby accelerating the
493 decomposition and transformation of organic matter and intensifying soil respiration.
494 Additionally, soils under green manure treatments exhibited lower annual average
495 temperatures compared to the control, indicating that intercropping with green manure
496 moderated surface soil temperatures and reduced daily temperature fluctuations. This

497 effect was particularly pronounced in summer, when green manure not only reduced
498 inter-row CO₂ emissions but also improved the microclimatic conditions of the tea
499 plantation.

500 Traditional CO₂ flux measurements in tea plantations have mostly focused only on
501 tea rows, often neglecting inter-row soil emissions (Yao et al., 2015; Chen et al., 2021).
502 In this study, static chambers were parallelly employed to measure CO₂ fluxes in tea
503 rows and inter-row areas, enabling a more accurate understanding of CO₂ emissions.
504 Results showed that inter-row CO₂ fluxes were significantly higher than those in tea
505 rows ($p < 0.05$), accounting for 52.6%, 57.3%, and 60.8% of the annual cumulative CO₂
506 emissions under CK, HM, and SM treatments, respectively. These findings emphasize
507 the substantial contribution of inter-row zones to overall CO₂ emissions. This
508 discrepancy is due to differences in management intensity: fertilization and tillage are
509 commonly performed in inter-row areas, while the soil beneath tea canopies
510 experiences minimal disturbance (Hirono and Nonaka, 2012). Moreover, pruning
511 residues often accumulate in inter-row zones, further intensifying microbial activity and
512 CO₂ emissions in these areas. The cumulative CO₂ emissions under HMG and SMG
513 treatments were significantly lower in the second year. The decrease in emissions can
514 be attributed to the gradual attenuation of the carbon priming effect induced by soil
515 disturbance during the initial experimental phase (Zhou, 2025), coupled with the long-
516 term positive effects of green manure on enhancing soil physical structure and
517 ecosystem stability (Gui et al., 2024). The increase in green manure biomass in the
518 following year indicates that the green manure system is transitioning from an initially
519 disturbed and unstable state toward a more productive and
520 carbon-sequestration-enhanced stable state (Figure A1). This trend not only reflects the
521 improved functioning of the soil ecosystem but also serves as an important driver for
522 further carbon sequestration, contributing significantly to the reduction in inter-row
523 CO₂ emissions observed in the following year. Therefore, long-term and systematic
524 monitoring of inter-row soil CO₂ emissions is essential for accurately assessing the
525 carbon dynamics and mitigation potential of tea plantation ecosystems.

526

527 **4.3 Differences in Environmental Drivers**

528 Soil CO₂ fluxes are regulated by multiple environmental factors, including
529 photosynthetic activity or vegetation productivity (Tang et al., 2005), and soil properties
530 such as temperature and moisture (Liu et al., 2023; Widanagamage et al., 2025). Among
531 them, temperature is widely recognized as a primary driver of seasonal variation in soil
532 respiration (Lang et al., 2017). Our results showed that CO₂ fluxes in both tea rows and
533 inter-row areas were significantly correlated with soil and air temperatures under
534 different green manure treatments (Fig. 7). In addition, carbon and nitrogen
535 transformation processes driven by microorganisms are closely coupled. Nitrification
536 and denitrification alter NO₃⁻-N and NH₄⁺-N levels, thereby influencing soil
537 physicochemical properties and microbial activity. As a result, CO₂ emissions exhibit
538 significant positive correlations with nitrogen mineralization, denitrification, and N₂O
539 emissions (Dai et al., 2020). This carbon–nitrogen coupling may interact with the
540 distinctive nutrient uptake characteristics of tea plants, which are ammonium-preferring
541 species with rapid NH₄⁺ assimilation (Xin et al., 2024). In our study, CO₂ fluxes under
542 the CKG treatment were positively correlated with NH₄⁺-N content (Fig. 9). NH₄⁺-N
543 levels under this treatment (71.20 mg·kg⁻¹) were significantly higher than in the SMG
544 and HMG treatments, whereas the corresponding soil pH value (3.97) was significantly
545 lower ($p < 0.05$). This concurrent high NH₄⁺-N level and strong acidification is due to
546 ammonium accumulation under conventional fertilization and subsequent H⁺ release
547 during nitrification (Chen et al., 2021). By contrast, the soil pH under green manure
548 intercropping treatments increased by 0.02–0.11 units compared to the CK treatment
549 (Fig. 6i), suggesting that root exudates and organic matter inputs from green manure
550 buffered soil acidification by reducing H⁺ release during NH₄⁺ nitrification. Moreover,
551 the NO₃⁻-N concentration in the SMG treatment (14.56 mg·kg⁻¹) was significantly
552 higher than that in other treatments (Fig. 6e), due to the high biomass of *Vulpia myuros*
553 C., which may reduce nitrate losses via runoff or leaching. Its active root system also
554 improved soil aeration, inhibiting denitrification under anoxic conditions.

555 SOC content reflects the dynamic balance between organic matter inputs and
556 decomposition (Mo et al., 2024). In our study, SOC levels in the HMT and SMT

557 treatments were higher than those in the CKT treatment, while their cumulative annual
558 CO₂ emissions were lower. This indicates that increasing SOC storage can help mitigate
559 greenhouse gas emissions, consistent with findings by Han et al. (2022). However, the
560 HMG and SMG treatments exhibited much higher SOC levels than CKG, while their
561 cumulative CO₂ emissions exceeded those of CKG. This implies that once SOC
562 accumulation surpasses a certain threshold, the excess carbon supply may stimulate
563 microbial activity and subsequently increase CO₂ emissions (Lim and Choi, 2014).
564 Interestingly, recent studies reveal that SOC thresholds can modulate the impact of
565 nitrogen fertilization on carbon sequestration. In SOC-poor soils, nitrogen inputs tend
566 to promote carbon accumulation and soil aggregation, enhancing SOC storage.
567 Conversely, in SOC-rich soils, nitrogen fertilization may enhance microbial metabolic
568 efficiency and increase microbial residue production (Ling et al., 2025). Studies in
569 different climatic zones of China have revealed that SOC thresholds are influenced by
570 factors such as climate and soil type. In the maritime monsoon climate zone, dual
571 thresholds for NO₃⁻-N and extractable iron (Fe) have been identified, beyond which
572 their marginal effects on SOC shift significantly. In the continental monsoon climate
573 zone, SOC content increases markedly once a critical threshold of TN is exceeded (Cui,
574 2025). Additionally, research in alpine ecosystems has shown that SOC components
575 vary along elevation gradients and exhibit distinct thresholds (Zhang, 2025). These
576 insights provide a new perspective for interpreting our results and highlight the
577 importance of identifying threshold values under multifactorial interactions to better
578 assess their effects on CO₂ emissions.

579 In tea rows, excessively high soil C/N ratios may result in nitrogen limitation,
580 thereby inhibiting rapid decomposition of organic matter and reducing CO₂ fluxes.
581 Green manure, as a fresh plant residue with a relatively low C/N ratio, can be rapidly
582 decomposed by soil microbes after incorporation, thus maintaining or enhancing SOC
583 levels (Li et al., 2024), which aligns with our observations (Fig. 7d). MBC and MBN
584 are generally considered closely linked to SOC (Gao et al., 2022). However, in our
585 study, a significant positive correlation between MBC and SOC was only observed in
586 the CKT treatment. The lack of correlation under green manure treatments may be due

587 to the rapid and excessive input of exogenous carbon, which complicates the
588 relationship between these variables. No significant differences in MBC and MBN
589 levels were found among green manure treatments, and both showed weak correlations
590 with CO₂ fluxes (Fig. 8), suggesting that MBC and MBN are not key drivers of CO₂
591 emissions in tea plantations.

592

593 **5 Conclusion**

594 This study revealed the regulation of CO₂ fluxes in tea plantations under different
595 green manure intercropping treatments. Green manure significantly influenced CO₂
596 flux dynamics, with pronounced seasonal variations, higher fluxes in summer and
597 autumn and lower fluxes in spring and winter. CO₂ emissions from inter-row areas were
598 consistently higher than those from tea rows. CO₂ fluxes in the SM and HM treatments
599 were significantly lower than in the CK treatment within tea rows, while the opposite
600 trend was observed in inter-row areas, suggesting distinct spatial responses to green
601 manure intercropping. Over the observation period, the HM and SM treatments reduced
602 CO₂ emissions from tea rows by 7.1%–7.9% compared to the CK, while increasing
603 inter-row emissions by 12.7%–28.9%. Inter-row CO₂ emissions accounted for 52.6%,
604 57.3%, and 60.8% of the annual cumulative CO₂ fluxes under the CK, HM, and SM
605 treatments, respectively. These findings highlight the importance of incorporating
606 spatial emission weighting into carbon accounting for agricultural ecosystems.

607 The observed spatial differences in CO₂ fluxes were closely related to variations
608 in SOC content. Our findings suggest the existence of a critical SOC threshold that
609 determines whether CO₂ emissions increase or decrease. Future research should focus
610 on quantifying such thresholds under multi-factor interactions to better assess their
611 impacts on greenhouse gas dynamics. Continuous green manure intercropping over two
612 years significantly reduced inter-row CO₂ emissions, and the HM treatment suppressed
613 fertilization-induced emission peaks. These findings demonstrate that green manure
614 intercropping, particularly mixed legume and non-legume combinations, can
615 effectively alter the spatial pattern of CO₂ emissions and mitigate carbon losses.
616 Moreover, this practice provides significant co-benefits like improved soil aeration and

617 fertility, reduced chemical fertilizers, weed suppression, and promoted tea plant growth,
618 thereby offsetting the extra costs in labor and seeds. Therefore, green manure
619 intercropping emerges as a practical and multifunctional strategy for reducing carbon
620 emissions in agroecosystems.

621

622 **Data availability**

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

625

626 **Author contributions**

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

632

633 **Competing interests**

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

635

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867 **Tables and Figures**868 **Table 1.** Initial basic physicochemical properties of the six treatments.

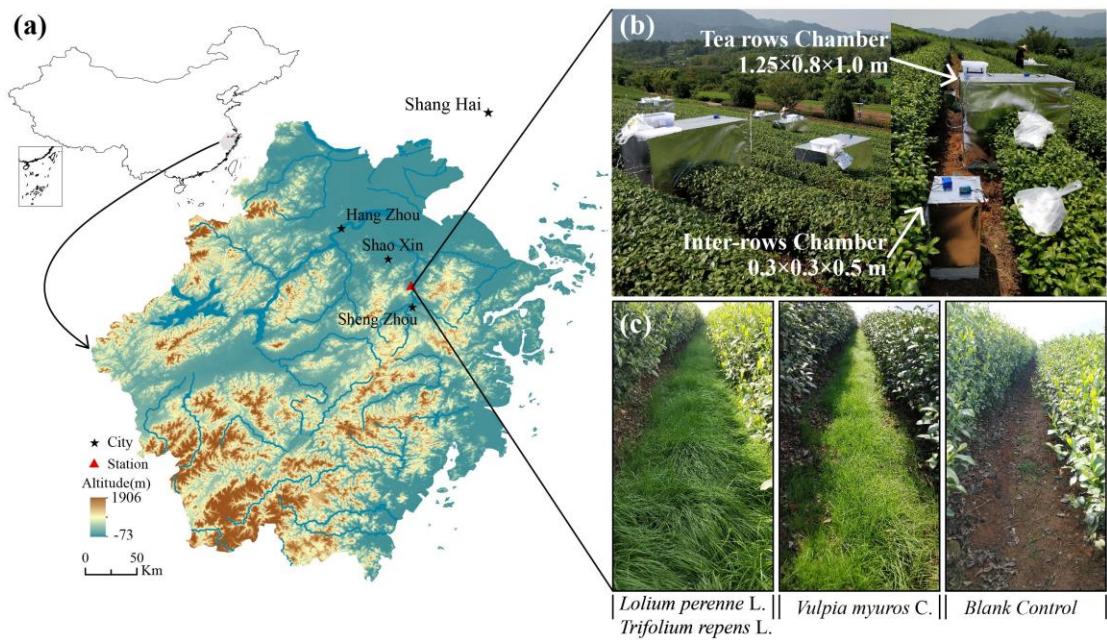
Tpye	pH	$\text{NH}_4^+ \text{-N}$ ($\text{mg} \cdot \text{kg}^{-1}$)	$\text{NO}_3^- \text{-N}$ ($\text{mg} \cdot \text{kg}^{-1}$)	TN ($\text{g} \cdot \text{kg}^{-1}$)	TC ($\text{g} \cdot \text{kg}^{-1}$)	SOC ($\text{g} \cdot \text{kg}^{-1}$)
CKT	4.25 ± 0.06^a	29.67 ± 7.50^b	5.01 ± 2.02^b	2.16 ± 0.16^{ab}	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 ± 8.93^b	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

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

872 **Table 2.** Seasonal variation in CO₂ fluxes from tea rows and inter-row zones under
 873 different green manure intercropping treatments.

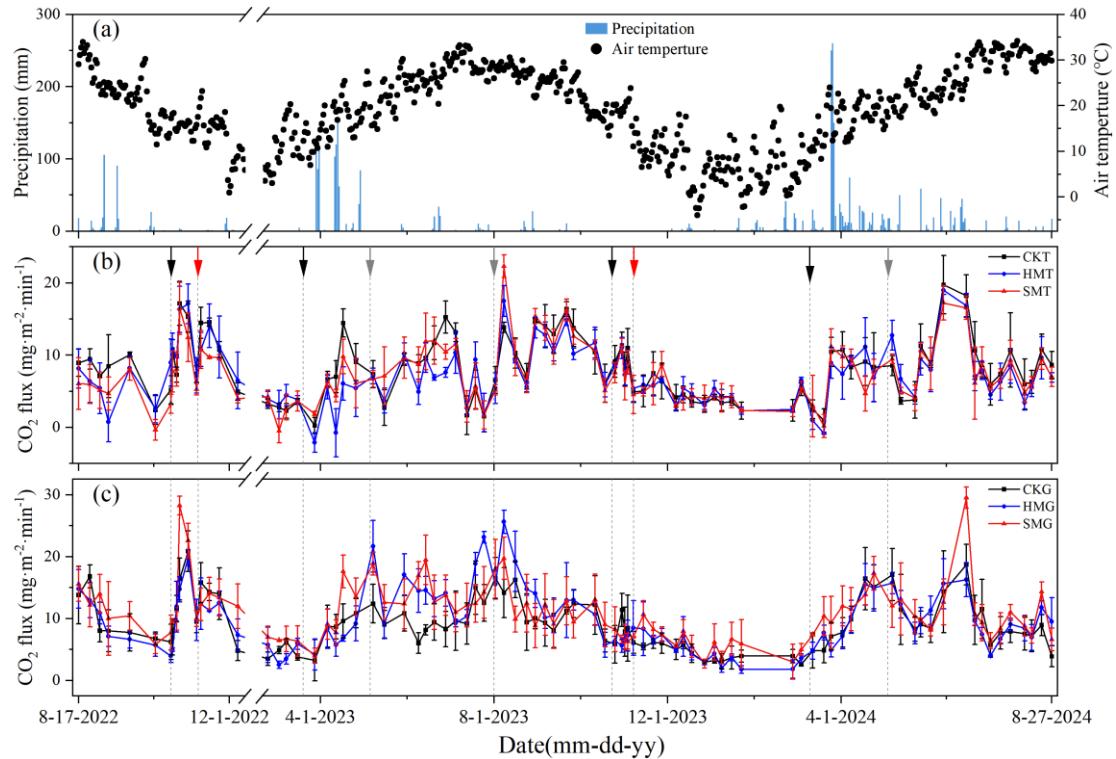
Type	Spring (mg·m ⁻² ·min ⁻¹)	Summer (mg·m ⁻² ·min ⁻¹)	Autumn (mg·m ⁻² ·min ⁻¹)	Winter (mg·m ⁻² ·min ⁻¹)
CKT	6.46±0.58 ^{bcd}	9.66±0.49 ^a	9.93±0.68 ^a	3.60±0.36 ^f
HMT	5.65±0.44 ^{def}	7.97±0.39 ^{abc}	9.21±0.46 ^a	4.10±0.43 ^{ef}
SMT	6.05±0.39 ^{cde}	8.99±0.34 ^a	8.47±0.30 ^{ab}	3.71±0.37 ^f
CKG	8.83±1.14 ^{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}

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



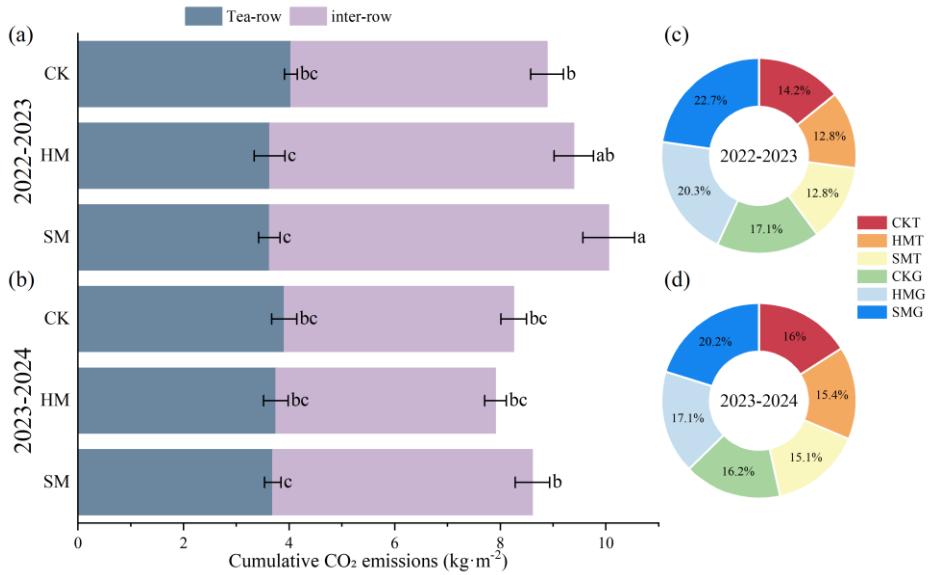
877

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



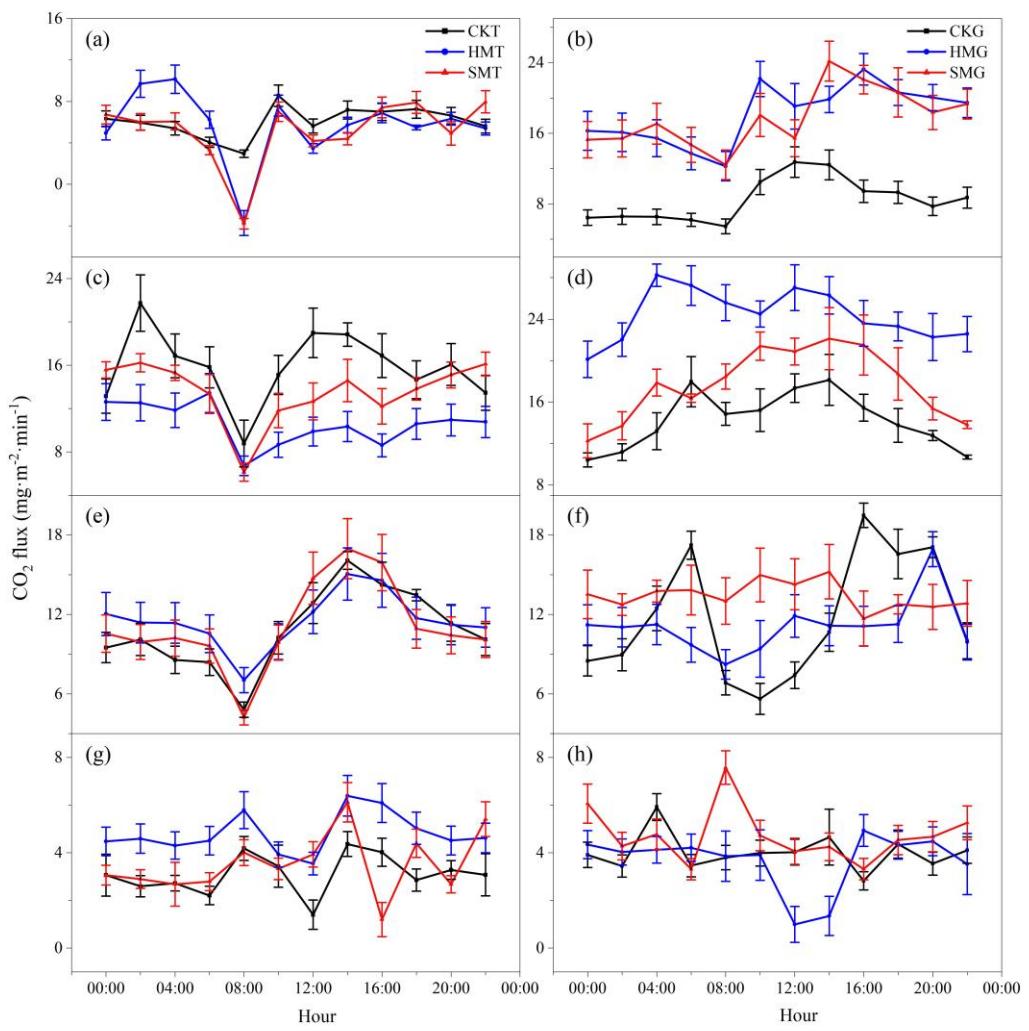
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883 **Figure 2.** Dynamics of (a) air temperature and precipitation, (b) CO₂ fluxes from tea
 884 rows, and (c) CO₂ fluxes from inter-rows during the observation period (2022–2024).
 885 Black, green and orange arrows represent the timings of fertilization, grass planting and
 886 tea pruning, respectively. Flux data are presented as mean \pm SE. CK for control; SM
 887 and HM for intercropping types, T for tea row, G for inter-row.



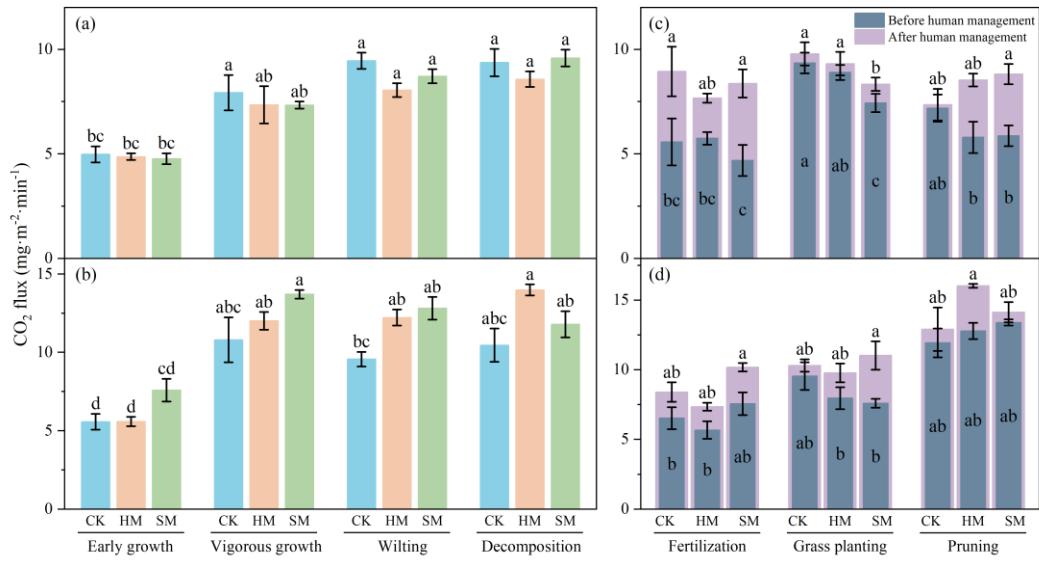
888

889 **Figure 3.** (a, b) Annual cumulative CO₂ emissions from tea rows and inter-rows under
890 different green manure intercropping treatments; (c, d) contribution of tea rows and
891 inter-rows to total annual CO₂ emissions under each treatment. Data shown are means
892 \pm SE. Different superscript letters denote statistically significant differences ($p < 0.05$).
893 CK for control; SM and HM for intercropping types, T for tea row, G for inter-row.



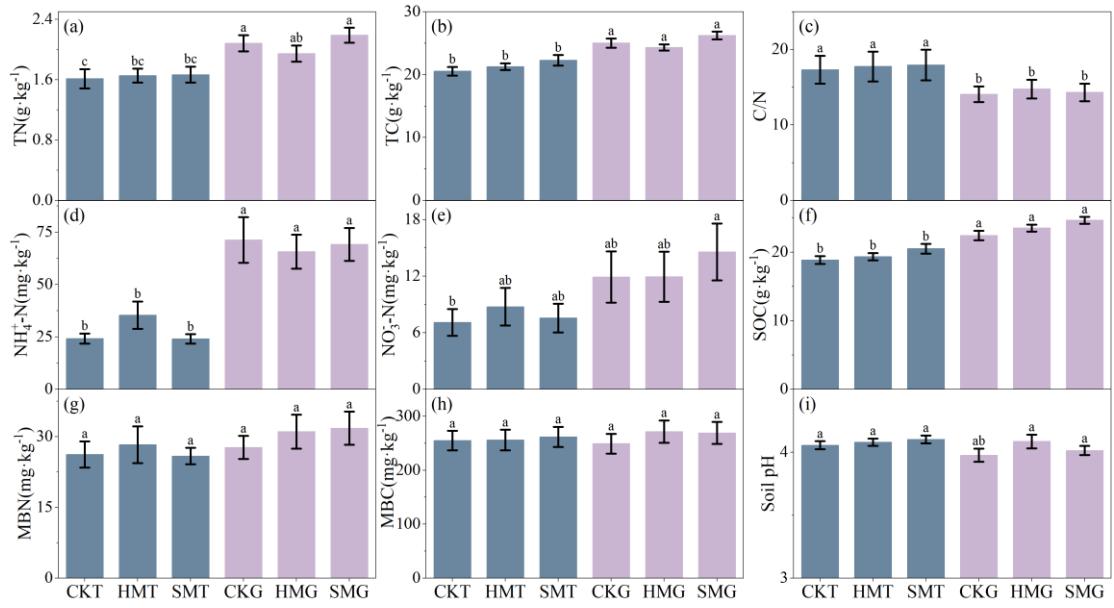
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895 **Figure 4.** Diurnal variation in CO_2 fluxes from (a, c, e, g) tea rows and (b, d, f, h) inter-
 896 row zones under different green manure intercropping treatments across seasons: (a–b)
 897 spring, (c–d) summer, (e–f) autumn, and (g–h) winter.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



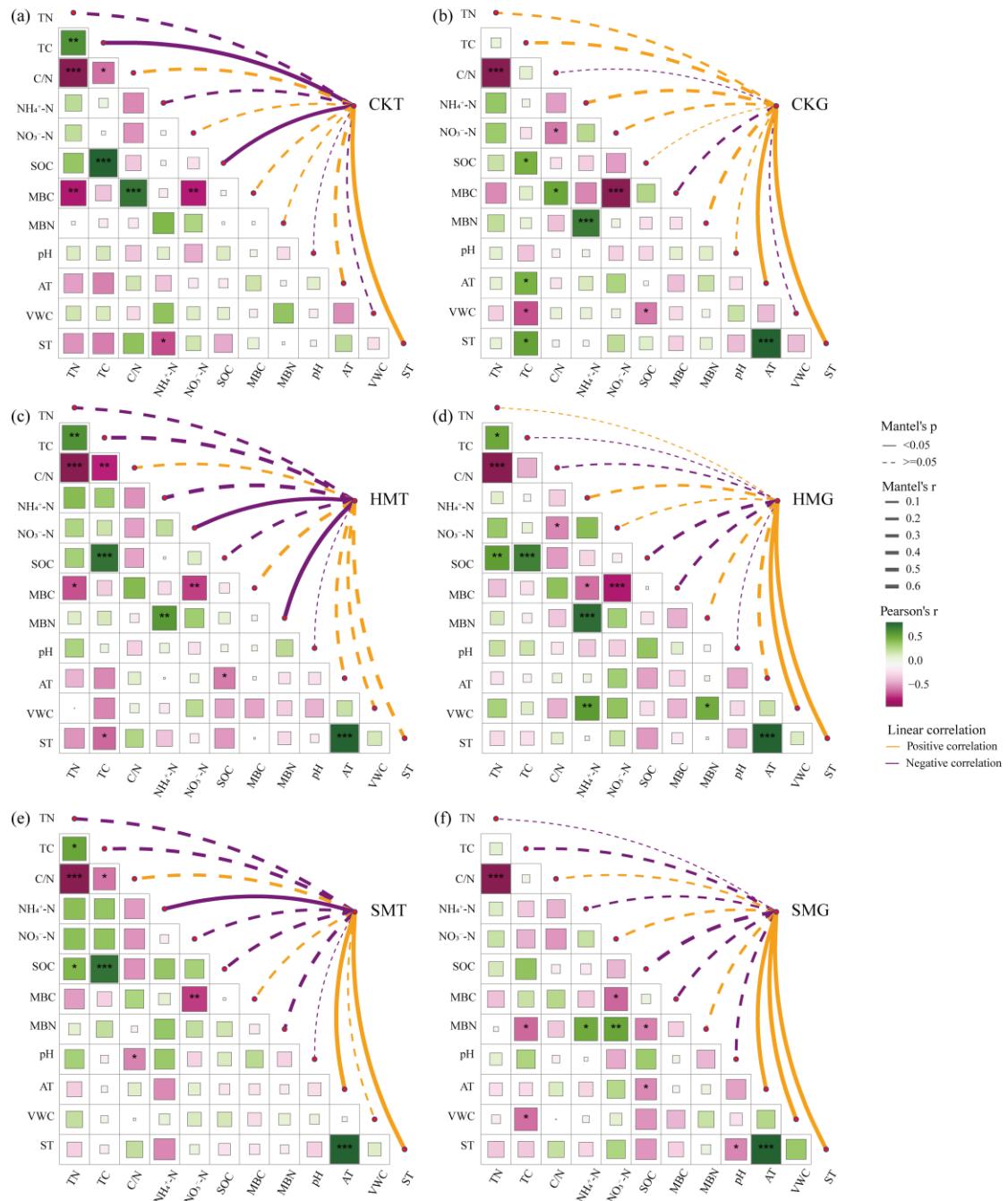
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900 **Figure 5.** Temporal dynamics of CO₂ fluxes under green manure (a, b) growth stages
 901 and (c, d) management events in tea plantations. Growth stages include: early growth
 902 (mid-November to early April), vigorous growth (mid-April to late May), wilting (early
 903 June to late July), and decomposition (August). CK for control; SM and HM for
 904 intercropping types, T for tea row, G for inter-row.



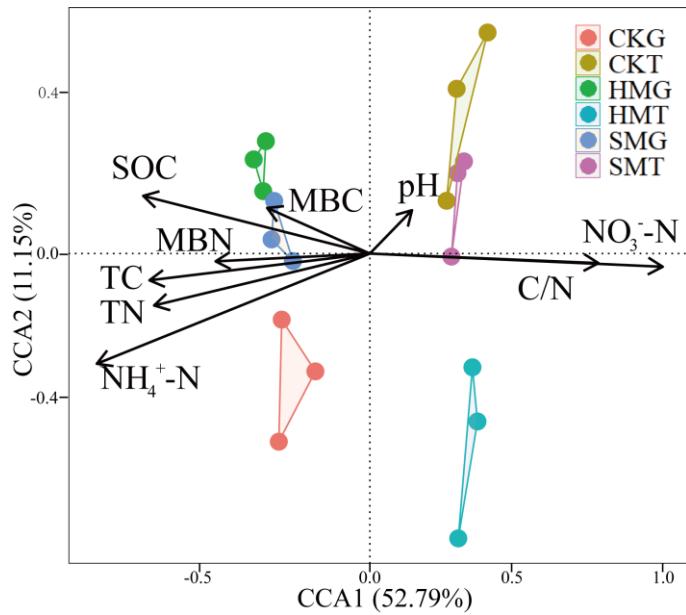
905

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



909

910 **Figure 7.** Pairwise correlations between environmental factors and their relationships
 911 with CO₂ fluxes under different green manure treatments (* $p < 0.05$, ** $p < 0.01$, *** p
 912 < 0.001). CK for control; SM and HM for intercropping types, T for tea row, G for inter-
 913 row.



914

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

Appendix 1

The CO₂ flux was calculated using the following equation:

$$3 \quad F = \rho_0 \times \frac{P}{P_O} \times \frac{T_O}{T+T_O} \times \frac{V}{M} \times \frac{\Delta c}{\Delta t} \quad (1)$$

where F is the CO_2 flux ($\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$); ρ_0 is the density of CO_2 under standard conditions ($1.98 \text{ kg} \cdot \text{m}^{-3}$); P_0 and T_0 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.

10 First, the raw CO₂ concentration readings were calibrated with standard gases.
11 Then, linear regression was performed to fit their rate of change over time. Finally, CO₂
12 flux was calculated using the standard flux formula by incorporating the chamber
13 volume, base area, and the measured atmospheric pressure and temperature.

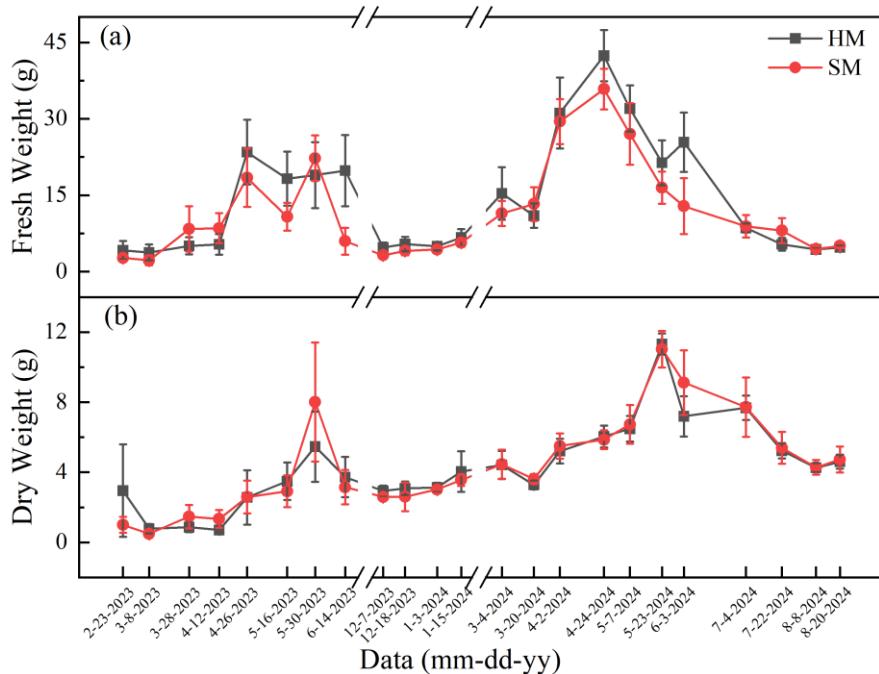


Figure A1. Temporal trend of green manure indexes