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Hao Liang, Jin Fu and Guopeng Zhou
contributed equally to this work.

Sustained Green Manure-Rice Rotations Can Mitigate Methane Emissions by Enhancing Microbial Methane Oxidation in Southern China

Hao Liang¹, Jin Fu¹, Guopeng Zhou^{2,3}, Jiguang Feng¹, Qian Zhu¹, Pete Smith⁴, Benoît Gabrielle⁵, Tao Li⁶, Susanne Schmidt⁷, Changxu Xu⁸, Jia Liu⁸, Jun Nie⁹, Ji Wu¹⁰, Mingjian Geng¹¹, Fei Wang¹², Yuting Liang¹³, Weidong Cao³, and Feng Zhou^{1,14} 

Key Points:

- CH₄ emissions decrease with increasing GM rotation year due to the decrease in the ratio of soil CH₄ production to oxidation gene abundance
- The RegWHCNS model was improved to simulate and evaluate the impact of GM rotation on CH₄ emission of paddy soils in southern China
- Nearly 76% of the paddy rice areas in southern China can achieve reductions in CH₄ emissions through approximately 15 years of GM rotation

Supporting Information:

Supporting Information may be found in the online version of this article.

Correspondence to:

F. Zhou and W. Cao,
zhouf@pku.edu.cn;
caoweidong@caas.cn

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Author Contributions:

Conceptualization: Hao Liang, Jiguang Feng, Qian Zhu, Pete Smith, Benoît Gabrielle, Tao Li, Susanne Schmidt, Yuting Liang, Weidong Cao, Feng Zhou
Data curation: Hao Liang, Guopeng Zhou, Changxu Xu, Jia Liu, Jun Nie, Ji Wu, Mingjian Geng, Fei Wang, Weidong Cao

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¹State Key Laboratory of Water Disaster Prevention, Jiangsu Key Laboratory of Soil and Water Processes in Watershed, College of Geography and Remote Sensing, Hohai University, Nanjing, China, ²Anhui Province Key Lab of Farmland Ecological Conservation and Nutrient Utilization, College of Resources and Environment, Anhui Agricultural University, Hefei, China, ³State Key Laboratory of Efficient Utilization of Arable Land in China, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China, ⁴Institute of Biological & Environmental Sciences, University of Aberdeen, Aberdeen, UK, ⁵Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS, Palaiseau, France, ⁶International Rice Research Institute, Los Banos, Philippines, ⁷School of Agriculture and Food Sustainability, The University of Queensland, Brisbane, QLD, Australia, ⁸Institute of Soil & Fertilizer and Resources & Environment, Jiangxi Academy of Agricultural Sciences, Nanchang, China, ⁹Soil and Fertilizer Institute of Hunan Province, Hunan Academy of Agricultural Sciences, Changsha, China, ¹⁰Institute of Soil and Fertilizer, Anhui Academy of Agricultural Sciences, Hefei, China, ¹¹College of Resources and Environment, Huazhong Agricultural University, Wuhan, China, ¹²Institute of Soil and Fertilizer, Resources and Environment, Fujian Academy of Agricultural Sciences, Fuzhou, China, ¹³State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China, ¹⁴Laboratory for Earth Surface Processes, Institute of Carbon Neutrality, College of Urban and Environmental Sciences, Peking University, Beijing, China

Abstract Green manure (GM) enhances the ecological services in agricultural ecosystems, including soil health and carbon sequestration. However, its effect on regional methane (CH₄) emissions from paddy fields is unclear. Here we clarify the impacts of GM rotation by combining process-based modeling with microbial gene abundance information and coordinated distributed observations at 14 sites in southern China. We found that GM management, including application rate and rotation year, mainly affects CH₄ emissions in GM-rice systems by impacting soil biotic factors, which explain 78.4% of the variation ($p < 0.001$). The most influential factor is the ratio of soil CH₄ production to oxidation gene abundances ($R^2 = 0.510$; $p < 0.001$), which decreases with GM rotation year due to increased activity of methane-oxidizing soil microbes ($p < 0.001$), indicating that CH₄ emissions from GM-rice systems decrease with increased GM rotation year. By incorporating these microbial mechanisms as quantitative parameters in process-based model, we project that approximately 76% of the paddy rice areas in southern China, which have relatively low GM biomass and baseline CH₄ emissions, can achieve reductions in CH₄ emissions through nearly 15 years of GM crop rotation. This study indicates that CH₄ emissions from GM-rice rotations with appropriate GM application rate over the long term will not significantly increase, resolving the contradictions in previous research.

Plain Language Summary Green manure (GM), which is like a natural fertilizer, helps make farmland healthier and better at storing carbon. However, its impact on methane (CH₄) emissions, a powerful greenhouse gas that contributes to climate change, from rice fields has been unclear. We studied this by looking at how GM is used in different ways at various locations in southern China. We discovered that how we use GM, such as how much we use and how often we rotate crops, mainly affects CH₄ emission from rice fields by changing the soil CH₄ oxidizing microbes. The most important finding is that the longer GM is used in rotation with rice, the lower the CH₄ emissions. Our research shows that if we use GM properly for a long time, it won't make the CH₄ emission problem worse. In fact, it can help reduce the CH₄ emission from rice fields after about 15 years, which helps clear up past disagreements about this issue.

Formal analysis: Hao Liang, Changxu Xu, Jia Liu, Jun Nie, Ji Wu, Mingjian Geng, Fei Wang, Feng Zhou

Funding acquisition: Weidong Cao, Feng Zhou

Investigation: Hao Liang, Jin Fu, Guopeng Zhou, Changxu Xu, Jia Liu, Jun Nie, Ji Wu, Mingjian Geng, Fei Wang, Yuting Liang

Methodology: Hao Liang, Jin Fu, Guopeng Zhou, Jiguang Feng, Qian Zhu, Pete Smith, Benoît Gabrielle, Tao Li, Susanne Schmidt, Yuting Liang, Feng Zhou

Project administration: Weidong Cao, Feng Zhou

Resources: Guopeng Zhou, Changxu Xu, Jia Liu, Jun Nie, Ji Wu, Mingjian Geng, Fei Wang, Yuting Liang, Weidong Cao, Feng Zhou

Software: Hao Liang, Jin Fu

Supervision: Weidong Cao, Feng Zhou

Validation: Hao Liang, Jin Fu

Visualization: Hao Liang, Jin Fu

Writing – original draft: Hao Liang, Jin Fu, Feng Zhou

Writing – review & editing: Hao Liang, Jin Fu, Guopeng Zhou, Jiguang Feng, Qian Zhu, Pete Smith, Benoît Gabrielle, Tao Li, Susanne Schmidt, Yuting Liang, Weidong Cao, Feng Zhou

1. Introduction

Green manure (GM), also known as “cover crop”, refers to plants grown on seasonally and spatially fallow lands to provide multiple benefits to agroecosystems. Reported benefits include enhanced soil carbon sequestration (Jian et al., 2020; McClelland, Paustian, & Schipanski, 2021), improved soil structure (Zhang & Peng, 2021), reduced erosion (Du et al., 2022), and increased biological diversity (Daryanto et al., 2018; Lamichhane & Alletto, 2022). However, incorporating GM into rice plantation may considerably increase greenhouse gas (GHG) emissions, particularly methane (CH₄) emission from anaerobic paddy fields (Hwang et al., 2017; Lou et al., 2024; Sanz-Cobena et al., 2014), which has long hindered the promotion and application of GM.

Quantifying CH₄ emissions is central to evaluate the effects of GM rotation because N₂O makes a comparatively minor contribution (3.5%) to the non-CO₂ GHG emissions from rice paddies (Lou et al., 2024). GM is traditionally used in paddy fields to reduce the application of nitrogen (N) fertilizers, which can decrease usage by 20%–40% in southern China (Gao et al., 2023); however, its impact on CH₄ emissions remains a significant uncertainty. Here we address the current uncertainty regarding how GM rotation impacts CH₄ emissions from paddy fields, as reported values vary widely, ranging from a 25.3% emission reduction to a 613.6% increase (median = 44.7%, $n = 51$; Table S1 in Supporting Information S1). A limitation is that most studies are based on observations from one or 2 years, with few focusing on the impact of the observation period on CH₄ emissions. In field experiments conducted over 1–3 years, the increase in CH₄ emissions from GM rotations was significantly higher (–1.4%–613.6%, median = 65.6%, $n = 38$, Table S1 in Supporting Information S1) compared to longer-term studies of 4 years or more (–25.3%–116.7%, median = 25.7%, $n = 13$, Table S1 in Supporting Information S1). The pronounced variation in CH₄ emissions attributed to differences in GM biomass application rate (Haqee et al., 2015; Yang et al., 2022), the C/N ratio of GM biomass (Datta et al., 2013; Raheem et al., 2019), drainage (Janz et al., 2019; Tariq et al., 2017), and N management practices (Bhattacharyya et al., 2012). The impact of different types of GM on CH₄ emissions is related to their biomass and C/N ratio of GM (Datta et al., 2013; Haqee et al., 2015; Raheem et al., 2019; Yang et al., 2022). Both high biomass and a high C/N ratio tend to promote CH₄ emissions, while low biomass and a low C:N ratio do not necessarily increase CH₄ emission (Hou et al., 2022; Raheem et al., 2019). Milk vetch (*Astragalus sinicus* L.), the most representative GM crop in southern China, accounts for over 80% of all GM crops in the region (Gao et al., 2023). With its lower biomass and C/N ratio, it is more suitable for paddy field systems and may not increase CH₄ emissions (Hou et al., 2022; Raheem et al., 2019). Short-term studies are therefore unlikely to capture the extended effects of GM's sustained cultivation on soil function and its role in modulating CH₄ emissions. The central question our study addresses is why CH₄ emissions differ significantly between short-term and longer-term GM rotations and what factors drive the observed variations.

Three potential mechanisms explain the differences in CH₄ emission observed in short-versus long-term GM rotations. First, incorporating GM increases soil organic matter and supplies substrates for methanogenic microbes, leading to higher CH₄ emissions in short-term (Gao et al., 2023; Garland et al., 2021). Second, long-term GM rotations alter the abundance and community composition of methanogenic and methanotrophic microbes in the soil, directly influencing CH₄ production and its consumption potential (Kim et al., 2020; Schmidt et al., 2019; Vukicevich et al., 2016; Zhou et al., 2023). Third, the bio-tillage of GM roots can improve the soil physical structure by increasing porosity and diffusivity, which may enhance CH₄ oxidation (Zhang & Peng, 2021). While GM rotation effects on soil properties have been investigated and benefits documented (Koudahe et al., 2022), it is not well understood how GM regulates production and consumption of CH₄ by soil microbes. The lack of a mechanistic understanding of GM rotation means that crop modelers tend to dismiss the potential effects on CH₄ emissions from paddy fields, even as GM rotation is expected to increase in the future (Gao et al., 2023). While current models, including DayCent (McClelland, Paustian, Williams, & Schipanski, 2021), ECOSYS (Qin et al., 2023), STIC (Tribouillois et al., 2018), and WHCNS (Liang, Li, et al., 2022) implicitly consider SOC increases with continuous application of GM, they fail to incorporate microbial mechanisms governing CH₄ production and oxidation, thus cannot account for the temporal effects of GM rotation on CH₄ emissions. This knowledge gap hinders the quantification of long-term GM rotation impacts on CH₄ emissions and the optimization of paddy rice cropping systems.

Southern China accounts for over 80% of the national rice planting area and 85% of the country's rice CH₄ emissions (National Bureau of Statistics of China, 2023; Qian et al., 2023; Tang et al., 2022). More than one-third of rice paddies in this region are fallow in winter, presenting a large potential for GM application and CH₄

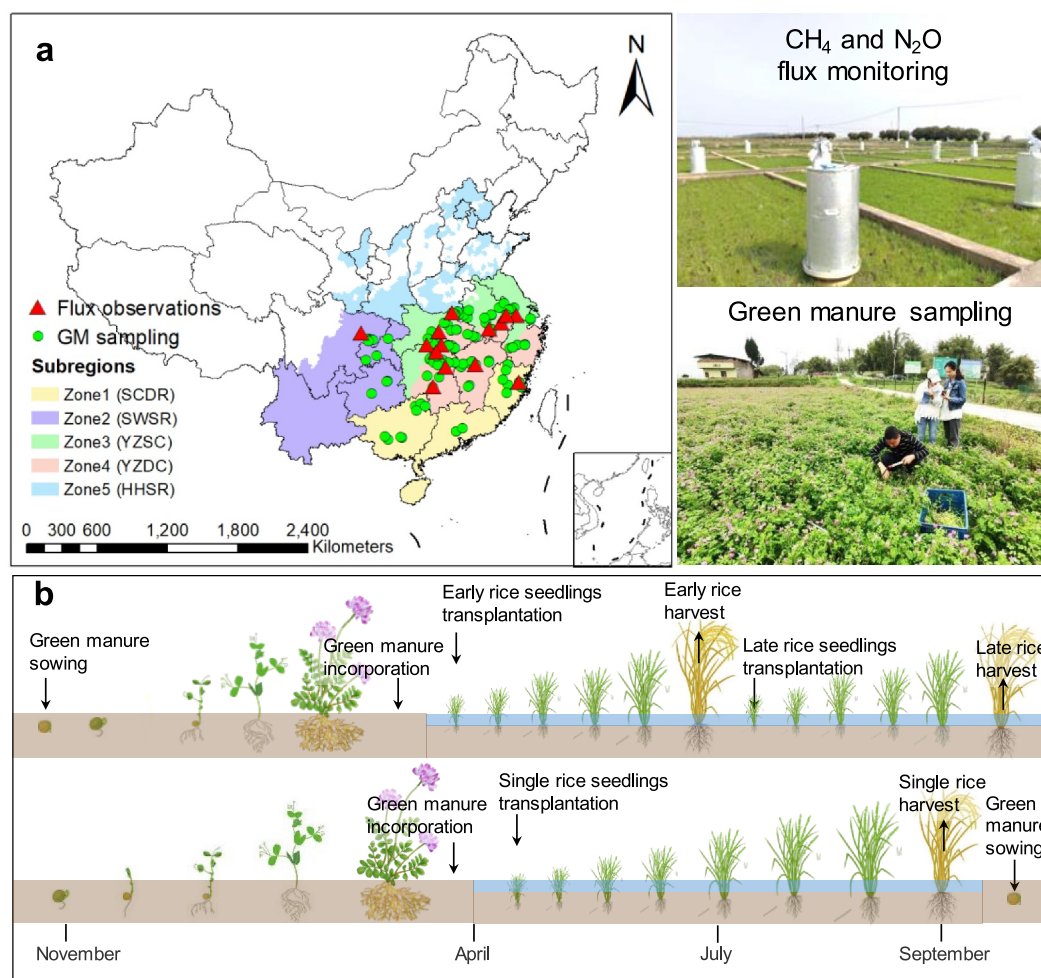


Figure 1. Locations of experimental sites (a) and timeline of field management of GM-rice systems (b). SCDR, South China double rice region; SWSR, Southwest single rice region; YZSC, Yangtze River middle and lower reaches single rice region; YZDC, Yangtze River middle and lower reaches double rice region; HHSR, the Huang-Huai-Hai single rice region.

mitigation (Gao et al., 2023). In this region, we established a coordinated network of distributed observations across 14 sites that are part of the National Green Manure Industry Technology System. Our study specifically focused on monitoring the CH_4 emissions from GM-rice and rice-fallow rotations across a range of GM rotation periods, spanning from 1 to 35 years. By integrating field observation, real-time quantitative PCR, and model simulations, we aimed to: (a) identify the key drivers of CH_4 emissions variations in GM-rice systems; (b) improve a process-based model by explicitly incorporating microbial processes governing CH_4 production and oxidation; and (c) evaluate the impact of GM rotation periods on CH_4 emissions in southern China.

2. Data and Methods

2.1. Study Area

Southern China locating in the warm temperate and subtropical zones, can be divided into five rice cultivation zones (Figure 1a) (Liao, 2010). The rotation of GMs, especially using legumes such as Milk Vetch (*Astragalus sinicus* L.), is one of the main cropping systems (more than 1.8 million hectares in 2020), accounting for nearly 10% of rice planting area in southern China (Gao et al., 2023). The GM crops are planted from late September to early October, and their biomass is incorporated into the soil when they reached the full flowering stage (late April to early May), about 2 weeks before rice seedlings transplantation (Figure 1b). The growing season of single rice lasts from early May to late September. Early rice is transplanted in late April and harvested in mid-July, followed by late rice's transplantation in mid-July and harvest in early October.

2.2. Flux Observations for GM-Rice and Fallow-Rice Systems

CH₄ fluxes were measured from 14 distinct experimental sites throughout a complete annual cycle, encompassing the growing seasons of rice, GM, and fallow seasons for up to 35 years (Figure 1a, Table S2 in Supporting Information S1). Fluxes were measured using a closed static chamber and gas chromatography technique (Yao et al., 2013; Zheng & Wang, 2017). In each plot, we chose a typical area and embedded a stainless steel base chamber (0.25 m² × 0.25 m height) to a depth of 15 cm into the soil. Top chambers were designed to fit exactly to the base chamber, with an adjustable height of 0.5 m before jointing stage and then 1.0 m to match plant height. A watertight seal at the interface between the frame and the chamber ensured the chamber's gas-tightness. The chamber bases remained in place throughout the study period, except for essential agricultural activities, while top chambers were temporarily installed during the gas flux measurements.

To measure CH₄ fluxes, five gas samples were taken from the chamber's headspace using 50 ml plastic syringes at 0, 10, 20, 30, and 40 min after covering. The concentrations of CH₄ were analyzed using gas chromatography (Agilent 7890A, Agilent Technologies, the United States). The fluxes were determined by measuring the linear and nonlinear changes in gas concentrations within the headspace of the sealed chamber over a period of time (Zheng & Wang, 2017). In general, measurements were taken every 3–5 days during the rice growth season, while a 10-day interval was used during the fallow and GM growth season. To minimize bias from diurnal temperature fluctuations, the single flux measurement was consistently taken between 09:00 a.m. and 11:00 a.m., when soil temperatures approximated the daily mean (Yao et al., 2013). A total of 7,488 daily flux data were measured from these 14 observational sites. Cumulative CH₄ emissions were estimated using linear interpolation to derive daily CH₄ fluxes between measurement intervals. The resulting data set comprised 78 site-years of flux observations from both GM-rice and fallow-rice systems. Site-specific soil properties (measured at 20 cm depth) were analyzed to identify key factors influencing GHG emissions (Table S3 in Supporting Information S1).

2.3. Microorganism Analysis Relating Methane Production and Oxidation

For each site, bulk density samples were collected using a soil auger at a depth of 0–20 cm with three replicates after harvest. Quantitative polymerase chain reaction (qPCR) was adopted to quantify the abundance of methanogenic archaea (*mcrA*), aerobic methanotrophs (*pmoA*), anaerobic methane-oxidizing bacteria (NC10 phylum), and sulfate reducing bacteria (*dsrA*) (Bridgham et al., 2013). Among these, the NC10 phylum and sulfate-reducing bacteria were mediated nitrate- and sulfate-dependent anaerobic oxidation of methane pathway, respectively (Zhou et al., 2020). The primer sets ml-F/ml-R, A189/mb661, qP1F/qP1R, and *dsrA*-290-F/RH3-*dsrR* were used to target the *mcrA*, *pmoA*, N10 phylum, and *dsrA* genes, respectively. Detailed information about the primers and qPCR conditions is listed in Table S4 of Supporting Information S1.

2.4. Spatiotemporal Variabilities of GM Biomass

To establish a driving data set for the regional model, we collected: (a) GM biomass, carbon and nitrogen content via a field survey and indoor analysis from 13 provinces. A total of 85 sampling points (with 5 duplicates) were chosen to assess the regional GM biomass (Figure 1a). Five plots (1 × 1 m) were randomly selected at each sampling point to measure the biomass (aboveground and belowground), carbon, and nitrogen content of the GM crops during their full-flowering stage, before being incorporated into the soil. (b) Soil samples (0–20 cm) were collected simultaneously to analyze the physical and chemical properties, that is, soil texture, organic carbon, total N (Table S5 in Supporting Information S1). (c) We also conducted questionnaire survey among local farmers to collect data on the timing and rate of N fertilizer application.

To estimate GM biomass and application rate at a grid (10 × 10 km) level for each year, we used a Random Forest (RF) model, with GM biomass as the dependent variable and latitude, longitude, elevation, four climatic variables and five soil variables as independent variables (Table S6 in Supporting Information S1). We determined the optimal number of trees to grow ($n_t = 150$), the number of randomly selected features for node splitting ($n_f = 4$), and the number of observations at the terminal nodes of the trees ($n_{ob} = 2$) by minimizing the out-of-bag mean squared error for each specific model. The RF model was evaluated using a five-fold cross-validation technique. The data set was divided into five equal-sized subsets, where 80% was used for model training and the remaining 20% was used to predict GM biomass using the fitted model (Bo et al., 2022). To prevent biases caused by random subset division, the validation process was repeated 10 times to allow for potential subdivisions. Model performance was evaluated with the correlation coefficient (R^2) and root mean squared error (RMSE) between the

predicted and observed values. The RF model could explain 82.5% of the spatio-temporal variations in GM biomass (Figure S1 in Supporting Information S1).

2.5. Parameterization for Process-Based Modeling

The WHCNS model was used to simulate CH₄ fluxes in a rice paddy ecosystem under GM rotation. The model is a process-based agricultural ecosystem simulation platform that is widely used to quantify the effects of agricultural management practices (i.e., rotation, straw return, and green manure) on water, carbon and N cycles, and crop growth processes in agricultural ecosystems (Jiang et al., 2021; Xu et al., 2020; Zhang et al., 2022). The model consists of six main modules simulating: soil water and heat flows, soil carbon cycling, soil nitrogen transport and transformations, crop growth, and field management practices. It operates on a daily time step and is driven by meteorological data, crop biological parameters, and field management. The mineralization process of GM was simulated according to the LIXIM model (Clivot et al., 2017), which is a model specialized in simulating the mineralization of soil organic matter, allowing us to effectively simulate the mineralization rates of GM under different incorporation rates (Liang, Li, et al., 2022). WHCNS can be run at both the field and regional scales. The regional version of the model has been established with a database of soil, meteorology, and crop grids for agricultural ecosystems in China. Parallel computing is employed to achieve rapid regional simulations (Liang, Hu, et al., 2023). An automated parameter calibration module (PEST, a model-independent parameter estimation tool) (Doherty, 2004) was incorporated, enabling rapid calibration of model parameters, and greatly improving the efficiency of model calibration and validation (Liang, Li, et al., 2022, Liang, Hu, et al., 2023).

The model uses “anaerobic ball” theory to simulate CH₄ missions under a given soil moisture condition. Reductive reactions (methane production process) occur in the anaerobic part of the soil, while oxidative reactions (methane oxidation) occur in the aerobic part (Liang, Xu, et al., 2022). The anaerobic fraction is calculated as follows:

$$f_{\text{ana}} = \begin{cases} 0.01 & , \theta < \theta_f \\ 0.01 + 0.99 \frac{\theta - \theta_f}{\theta_s - \theta_f} & , \theta \geq \theta_f \end{cases} \quad (1)$$

where f_{ana} represents the anaerobic fraction (ranging from 0 to 1), θ denotes the soil moisture content ($\text{cm}^3 \text{cm}^{-3}$), θ_s and θ_f refer to the saturated soil water content and field capacity, respectively ($\text{cm}^3 \text{cm}^{-3}$). Methane-producing microorganisms generate CH₄ by utilizing dissolved organic carbon (DOC) as a source material. This biochemical process follows a Michaelis-Menten equation:

$$MP_a = MP_s \cdot \frac{c_{\text{DOC}}}{k_{m\text{DOC}} + c_{\text{DOC}}} \quad (2)$$

$$MP_s = \begin{cases} 0 & T_s \leq 0^\circ\text{C} \\ MP_{\text{max}} \cdot (Q_{10\text{-CH}_4})^{\frac{T_s-30}{10}} & 0^\circ\text{C} < T_s < 30^\circ\text{C} \\ MP_{\text{max}} & 30^\circ\text{C} \leq T_s \end{cases} \quad (3)$$

$$MP_{\text{max}} = m_p \cdot dz \cdot \rho \cdot f_{\text{ana}} \quad (4)$$

where MP_a and MP_s is actual and potential CH₄ production rate for each soil layer, respectively ($\text{mg kg}^{-1} \text{d}^{-1}$); $k_{m\text{DOC}}$ and c_{DOC} is the half-saturation constant and DOC concentration, respectively (mg kg^{-1}); T_s is soil temperature ($^\circ\text{C}$); dz is the thickness of the soil layer (m); ρ is soil bulk density (mg cm^{-3}); $Q_{10\text{-CH}_4}$ is the Q_{10} coefficient for CH₄ production (–), m_p is the soil specific potential CH₄ production rate for each layer at 30 $^\circ\text{C}$ ($\text{g g}^{-1} \text{d}^{-1}$). The process of CH₄ oxidation within aerobic fraction in each soil layer described as:

$$\text{MO} = \text{MO}_{\text{max}} \cdot (1.0 - f_{\text{ana}}) \cdot f_{\text{oxid}}(T_s) \cdot f(z) \quad (5)$$

$$\text{MO}_{\text{max}} = m_o \cdot dz \cdot \left(1 - \frac{\rho}{2.65}\right) \quad (6)$$

$$f_{\text{oxid}}(T_s) = \begin{cases} 0.0 & T_s < -1^\circ\text{C} \\ (T_s + 1) \cdot (Q_{10_oxi})^{(T_s - 20)/10} & -1^\circ\text{C} \leq T_s \leq 0^\circ\text{C} \\ (Q_{10_oxi})^{(T_s - 20)/10} & 0^\circ\text{C} < T_s \end{cases} \quad (7)$$

$$f(z) = \exp\left(-\frac{z}{z_t}\right) \quad (8)$$

where MO and MO_{max} is actual and potential CH_4 oxidation rate for each soil layer, respectively ($\text{mg m}^{-2} \text{d}^{-1}$); m_o is the soil specific potential CH_4 oxidation rate per soil porosity ($\text{mg m}^{-3} \text{d}^{-1}$). $f_{\text{oxid}}(T_s)$ and $f(z)$ serve as scaling factors that correspond to soil temperature and depth, respectively; z and z_t is soil depth and the e-folding depth of CH_4 oxidation, respectively (m).

We used LnR (natural logarithmic response ratio) as an effect size to characterize the impact of GM planting on CH_4 emissions in rice fields.

$$\text{LnR} = \ln\left(\frac{f_g}{f_f}\right) \quad (9)$$

where LnR is the effect size of CH_4 ; f_g and f_f is the fluxes of CH_4 from GM-rice and fallow-rice system, respectively (kg ha^{-1}).

Model parameters were calibrated using CH_4 flux data from the fallow-rice system in the first year, and then validated using flux data from the GM-rice system as well as the remaining data from fallow-rice system. The target of the model calibration was the daily CH_4 flux data. First, we applied the PEST parameter inversion algorithm to adjust the input parameters for potential CH_4 production rates (m_p ; Equation 4) and oxidation rates (m_o ; Equation 6) of the fallow-rice system, with the objective function being the sum of squared residuals between the simulated and measured CH_4 fluxes. Subsequently, with m_p and m_o values held constant for each site (Table S7 in Supporting Information S1), we evaluated the model's performance in predicting the effects of GM practices across GM rotation years and application rates. Second, the potential CH_4 oxidation rate (m'_o) for the improved dynamic model was calculated under the assumption that the gene abundances of *pmoA*, *N10* phylum, and *dsrA* adequately represent the parameter m'_o , which is supported by the activity of these microbial groups within the soil. Thus, the original static model was updated to a dynamic model by considering this parameter m'_o and the gene abundances of methane-oxidizing bacteria in proportions similar to those of the GM rotation year (Figures S3b and S3d in Supporting Information S1), following a linear correlation between the GM rotation years (GRY; yr) and potential CH_4 oxidation rates ($\ln(m'_o/m_o)$).

$$\ln(m'_o/m_o) = a \times \ln(\text{GRY}) + b \quad (10)$$

where m_o and m'_o represent potential CH_4 oxidation rate for static and dynamic models, respectively ($\text{mg m}^{-2} \text{d}^{-1}$). a and b are model coefficients that were determined through the PEST parameter inversion method, with the objective of optimally simulating CH_4 emission data across varying GM rotation years. Third, we calibrated the model's parameter of CH_4 production rates in the GM-rice system (m_p) using a straightforward linear regression approach. Since the gene abundance of *mcrA*, which is mainly associated with CH_4 production, exhibit minimal variations with the years of GM rotation (Figure S3 in Supporting Information S1), the effect of GRY was ignored and m_p was considered proportional to the production potential of fallow-rice systems (m'_p):

$$m_p = c \times m'_p \quad (11)$$

where m_p and m'_p represent potential CH_4 production rate for fallow-rice and GM-rice systems, respectively ($\text{g m}^{-1} \text{d}^{-1}$). c is a model coefficient reflecting the effects of GM application rates on CH_4 emissions (Figure 2e). By applying this dynamic parameterization method, we corrected the original model's insensitivity and biases concerning the different GM rotation years and GM application rates.

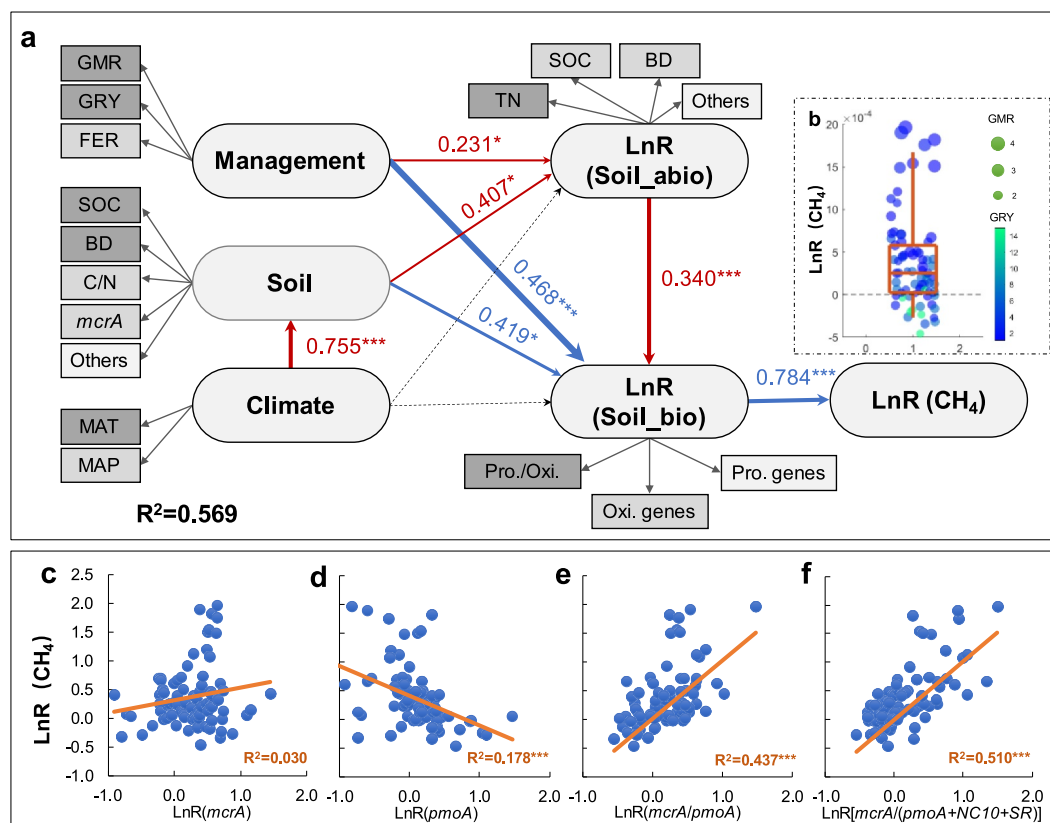


Figure 2. Key pathways and factors affecting the variability of CH₄ emission in the GM-rice system. Panel a presents the partial least square path models (PLSPM) that describe the key pathways and factors of LnR (CH₄). Panel b illustrates the variability of LnR (CH₄) under different management practices ($n = 78$, with each point representing the mean of three replications). Red and blue arrows represent negative and positive correlations, respectively; solid-line paths indicate significant effects, while dashed-line paths indicate non-significant effects; the symbols “*”, “***”, and “****” denote significance at the 0.05, 0.01, and 0.001 levels, respectively. In the rectangle, the deeper the color of a variable, the greater the corresponding loading. GMR refers to the GM application rate, GRY is the GM rotation year, Fer is the nitrogen application rate, SOC is soil organic carbon, C/N is the ratio of total carbon to total nitrogen in the soil, BD is soil bulk density, MAT is the annual mean air temperature, and MAP is the annual precipitation. The *mcrA*, *pmoA*, *NC10*, and *SR* are the abundance of methanogens, methanotrophs, *M. oxyfera* 16S rRNA, and *dsrA* genes, respectively. Pro. refers to the gene abundance related to soil methane production (*mcrA*), and Oxi. refers to the gene abundance related to soil oxidation (*pmoA*, *NC10*, and *SR*). The ratio of Pro./Oxi. represents the ratios of LnR (*mcrA/pmoA*) and LnR [*mcrA*/(*pmoA* + *NC10* + *SR*)]. Panels (c, d, e, and f) illustrate the relationships between LnR (*mcrA*), LnR (*pmoA*), LnR (*mcrA/pmoA*), and LnR [*mcrA*/(*pmoA* + *NC10* + *SR*)] with LnR (CH₄), respectively.

2.6. Process-Based Modeling for CH₄ Emissions at Regional Scale

It is imperative to establish a baseline by simulating the spatial and temporal distribution of CH₄ emission within the fallow-rice system before evaluating GM systems. We employed gridded data on CH₄ (EDGAR, 2023) emission fluxes from agricultural soils in 2010, to calibrate and evaluate the model in simulating the fallow-rice system (Baseline). The calibration was mainly conducted through the PEST in adjusting CH₄ production parameters (m'_p) and CH₄ oxidation parameters (m'_o) parameters, to find the best simulations for both mean and distribution of CH₄ (Figure S2 in Supporting Information S1). Then we calculate the CH₄ production parameters under different GM rotation years and application rates using Equations 10 and 11, involving the m_p under baseline conditions, the region and year specific GM biomass data. This process allows for the parameterization of the GM-rice model at the regional level for a long-term period, integrating the specific characteristics of GM practices within the modeling framework.

Finally, the resolution of the model region simulation was set at 10*10 km to simulate the spatial and temporal changes in GHG emissions from fallow-rice and GM-rice systems over 20 years (2000–2019). The RegWHCNS

model develops based on the WHCNS model, simulates at the grid scale, and integrates the HWSD database (FAO/IIASA/ISRIC/ISSCAS/JRC, 2012), which contains gridded data on soil organic matter, soil texture, bulk density, and pH value. The model can simulate the effect of a variety of agricultural management practices of irrigation, fertilization, and crop rotation conditions etc. on crop system. For the irrigation module, the model facilitates automatic watering by establishing the minimum and maximum water level thresholds (Jiang et al., 2021). As for fertilization, seeding and harvest dates, the study region has been divided into five distinct rice cultivation zones (Figure 1a), each configured according to local farming practice. These management details are provided in Table S8 of Supporting Information S1. Data sets required for regional simulation are as follows: (a) rice cultivation area in southern China (Luo et al., 2020); (b) soil property database (FAO/IIASA/ISRIC/ISSCAS/JRC, 2012); (c) the grid-scale daily meteorological data was interpolated using the inverse distance weighting method based on the station data from 2,474 national weather stations in China (<http://data.cma.cn/>); (d) The rice cultivar parameters and field management practices are based on the previous multi-point calibration results (Liang, Zhou, et al., 2023) and remain consistent across all subregions.

Four statistical indicators are used to evaluate model performance in simulating CH₄ and N₂O flux: correlation coefficient (Pearson's r), normalized root mean square error (NRMSE), Nash-Sutcliffe modeling efficiency (NSE), and index of agreement (IA). Among these, the values of r , NSE, and AI closer to 1 indicate better simulation results, while the NRMSE index expresses the percentage of the root mean square error to the mean; the lower the value, the higher the simulation accuracy.

3. Results

3.1. Key Pathways and Factors Affecting the Variability of CH₄ Emission

Climate, soil, and management are potential variables that can affect the CH₄ emissions of the GM-rice system by mediating the biotic and abiotic factors of the soil. Path analysis models indicate that GM management (GMR and GRY) influences LnR (CH₄) by affecting soil biotic (Soil_bio) and abiotic (Soil_abi) factors, representing the most critical impact pathways (Figure 2a). Among these, the impact of the Management on soil oxidation-related biotic factors was extremely significant ($p < 0.001$), and Soil_bio accounted for 79.8% of the variation in LnR (CH₄) ($p < 0.001$), indicating that the Management can influence CH₄ emissions by mediating soil methanotrophic microorganisms. A higher amount of GM application rate can increase the substrate (TN, SOC) for CH₄ production, thereby increasing the risk of CH₄ emissions in the system (Figure 2a); longer-term GM rotation year can enhance soil methane oxidation capacity, which helps to reduce the system's CH₄ emissions (Figure 2a). Under the influence of these factors, the LnR (CH₄) of the GM-rice system exhibits significant spatiotemporal variation characteristics (Figure 2b).

The abundance of the *mcrA* gene exhibits an extremely significant correlation with LnR (CH₄) ($p < 0.001$, Figure 2c), whereas the *pomA* gene shows no significant correlation (Figure 2d). However, the explanatory power of the abundance of either gene alone for LnR (CH₄) remains low ($R^2 < 0.178$). Ratios of *mcrA/pmoA* (or *mcrA/(pmoA + NC10 + SR)*) gene abundances serve as the valuable metric for estimating the potential CH₄ emissions (Jiang et al., 2022; Lee et al., 2014; Yuan et al., 2018). The variation in LnR (CH₄) of GM-rice system can be explained by ratios of *mcrA/pmoA* (by 43.7%, $p < 0.001$) and *mcrA/(pmoA + NC10 + SR)* (by 51.0%, $p < 0.001$; Figures 2e and 2f). These ratios decrease logarithmically with the increasing GM rotation years ($p < 0.01$; Figure S3 in Supporting Information S1), indicating a diminishing potential for CH₄ emissions with the long-term GM rotation. The declines in *mcrA/pmoA* (or *mcrA/(pmoA + NC10 + SR)*) ratios were predominantly driven by increases in abundance of *pmoA* and *SR* genes ($p < 0.01$; Figure S3 in Supporting Information S1), rather than decreases in *mcrA* gene abundance (Figure S3 in Supporting Information S1). It suggests that long-term GM crop rotations reduce CH₄ emissions by increasing the population of methanotrophic bacteria and thus increase CH₄ oxidation.

Under the influence of GM application rate and GM rotation year, significant differences in CH₄ emissions are observed in the GM-rice system. An appropriate incorporation rate and longer GM rotation years may not increase the CH₄ emissions of the GM-rice system, with LnR (CH₄) exhibiting negative values (Figure 2b). Although current models account for the impact of GMR on SOC or CH₄ production, they often overlook the temporal factor. Therefore, accurately assessing the CH₄ emissions of the GM-rice system requires the incorporation of the temporal factor into process-based models.

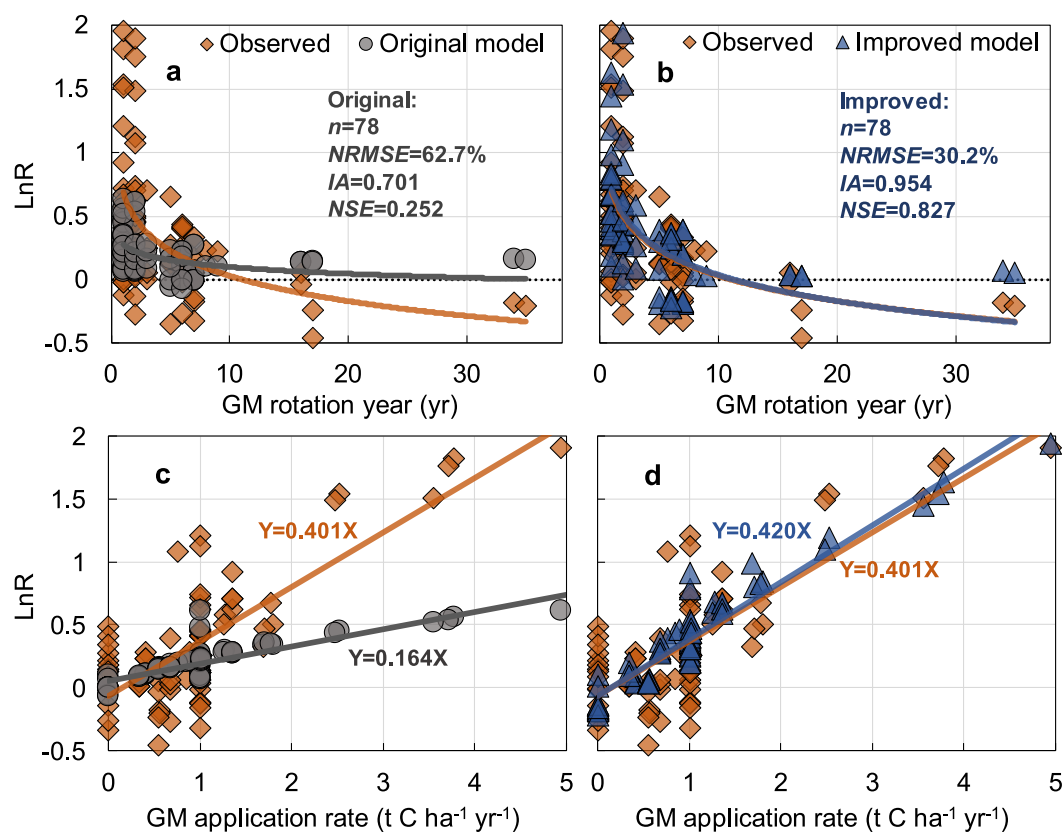


Figure 3. Response of the improved RegWHCNS model for modeling the effect size of CH_4 emission under different GM rotation years (a) and application rates (b).

3.2. Model Simulations for GHG Emissions Along Rotation Years

Based on the above microbial mechanisms, we have modified the processes of CH_4 oxidation and production within the WHCNS model to capture the spatiotemporal variations in CH_4 emissions. The model with the microbial processes was calibrated by the field observations of rice-fallow system in the first year, and then validated by flux observations from GM rotations and the remaining years of the fallow-rice system. The results show that the model, in contrast to that without the microbial processes, can reproduce the CH_4 missions of 13 site-years at daily timescale (NSE = 0.954, IA = 0.827; Figures S4 and S5 in Supporting Information S1) and at annual timescale (NSE = 0.914, IA = 0.795; Figure 3, Figure S6 in Supporting Information S1). Simulations from model without the microbial processes hardly capture the effect of GM rotation on CH_4 emissions reduction (Figure 3a), while the improved model simulated the turning point at 10-year of GM rotation that is close to field observations (Figure 3b). In addition, the improved model can also capture CH_4 emissions variations from different GM application rates, with comparable slope to observations of 0.40 (Figures 3c and 3d). These results indicate that the improved model can be used to accurately simulate CH_4 emissions under various combinations of GM rotation years and application rates at large scale.

3.3. Projection of GM Impacts on CH_4 Emissions

The CH_4 emissions hardly varied in the fallow-rice system from 2000 to 2019 (Figures 4a–4d), but significantly decreased with the growth of GM rotation years in the GM-rice system (Figures 4e–4h). Areas of the LnR values below zero accounted for 33.5% after a 5-year GM rotation to 78.5% after a 20-year GM rotation (Figure 4k). The largest LnR values occurred in the Middle and Lower Reaches of the Yangtze River Basin, where the greatest potential for GM applications and CH_4 emissions exists. The variable of R_y indicates the number of years of GM

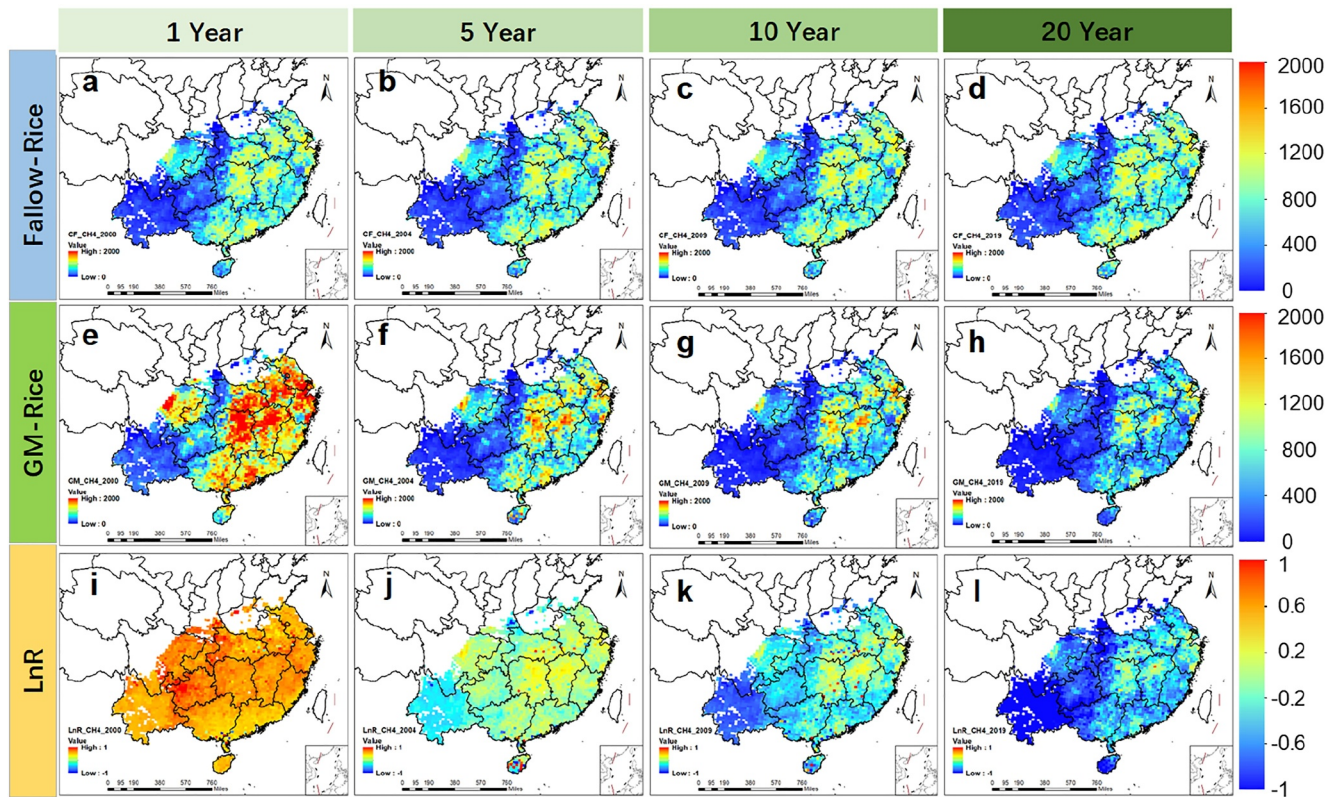


Figure 4. Spatio-temporal variability in CH_4 emission ($\text{kg ha}^{-1} \text{ year}^{-1}$) of fallow-rice and GM-rice systems in southern China.

rotation required for CH_4 emission to become lower than those in the fallow-rice system in this region (Figure 5a). On average, it takes 7 years for CH_4 emissions from GM systems to become lower than those from fallow-rice systems (Figure S7a in Supporting Information S1). This is primarily due to the gradual reduction of CH_4 emissions in the GM-rice system over time (Figure 4), as the total GM biomass in this region remained stable during the study period (range 2.63–2.98 $\text{t ha}^{-1} \text{ yr}^{-1}$; Figure S8 in Supporting Information S1).

Spatially, the R_y of the GM-rice system in southern China exhibits significant uncertainty (Figure 5a). The longest period is identified in the double rice cropping system in the Middle and Lower Reaches of the Yangtze River Basin (Figure S7e in Supporting Information S1). And the shortest period is observed in single rice cropping system in the Southwest region (Figure S7c in Supporting Information S1). Importantly, the highest GM biomass was found in the Middle and Lower Reaches of the Yangtze River Basin (4.5 t ha^{-1}) and an about 1.0 t ha^{-1} lower biomass in the southwestern region (Figure S8 in Supporting Information S1). Variables of latitude, precipitation, elevation, sunshine duration, temperature etc., are key factors affecting GM biomass production ($R^2 = 0.68$; Figure S1b in Supporting Information S1). The simultaneously highest GM biomass production and longest periods for reduction in CH_4 emissions needs careful evaluations of the suitability of GM systems in the Middle and Lower Reaches of the Yangtze River Basin.

In southern China, CH_4 emissions reached a plateau value after 15 years, with more than 76% of the areas exhibiting reductions in CH_4 emissions (Figure 5b). This highlights that the remaining 24% rice paddies hardly reduced CH_4 emissions through single GM practice. Rice planting patterns (rice zones), GM biomass, location, elevation, sunlight hour, soil organic carbon and bulk density are the main drivers, explained in a total of 78% of the R_y distribution (Figure 5c). Among these, subzones and GM are the most critical influencing factors, indicating that within similar rice planting regions, GM biomass is the most important factor affecting R_y . Interactions also have stronger explanatory power for R_y distribution, particularly those between meteorological/soil factors and GM biomass (Figure 5d), indicating that the spatial variation of R_y is driven by a combination of human activities, and geographical and meteorological conditions.

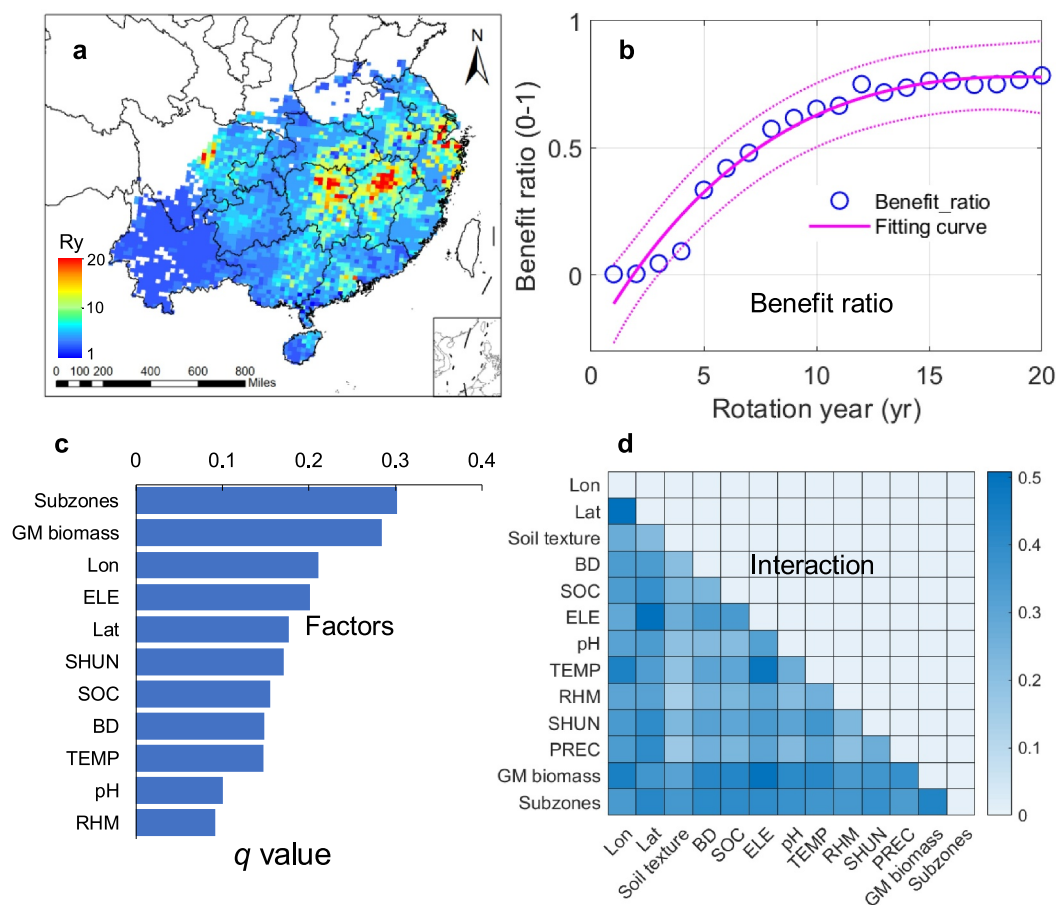


Figure 5. Spatial variations in the potential of CH₄ emission reduction and key driving factors. Panel a shows the number of years of GM cropping required to reduce CH₄ emissions from GM-rice systems below those from fallow-rice (Ry value), with higher values indicating longer periods of green manure cropping and lower values indicating shorter periods. Panel b shows the proportion of areas with LnR < 0 (i.e., CH₄ emissions from GM-rice systems are lower than those from fallow-rice systems) in southern China rice regions as the GM rotation years increase. Panel c shows the explanatory power (q value) of spatial variables calculated by Geodetector (Text S1 in Supporting Information S1) on Ry value, and Panel d shows the interaction of spatial variables.

4. Discussion

By integrating process-based modeling with field network observations, this study has elucidated the time-dependent relationship between the duration of GM crop rotation and CH₄ emissions from paddy fields in southern China. Our findings demonstrate that continuous GM crop rotation does not result in increased CH₄ emissions. The observed significant reduction in CH₄ emissions is associated with enhanced microbial methane oxidation, which is a consequence of prolonged GM crop cultivation and use. Additionally, our study offers a comprehensive, large-scale assessment of the impact of GM crop rotation on CH₄ emissions, demonstrating that it is a sustainable and climate-friendly agricultural practice in the paddy soils of southern China.

Soil CH₄ emissions are the final result of CH₄ production, transportation, and oxidation processes. Approximately 80% of CH₄ from methanogenic archaea is oxidized either aerobically or anaerobically before being released into the atmosphere (Bridgman et al., 2013; Conrad & Rothfuss, 1991; Frenzel et al., 1992). We provided evidence that the annual increment in the abundance of soil CH₄-oxidizing bacteria, which includes both aerobic and anaerobic strains, is the principal driver for the observed reduction in CH₄ emissions from the GM-rice system. Although GM-rice rotation has gradually increased the potential of soil CH₄ oxidation, the regular incorporation of GM biomass into soils provides additional substrates for methanogenic microbes. The concerns about increases in CH₄ emissions during these short periods are reasonable and necessary, since it results in a build-up of CH₄ in

the atmosphere before oxidation (Haque et al., 2015). This is especially a problem in double rice cropping areas of the Middle and Lower Reaches of the Yangtze River Basin (Zone4), where a longer period of GM rotation is needed to mitigate the initial spike in CH₄ emissions (Figure 5a). However, it is not a concern in the Southwest and Huang-Huai-Hai single rice region (Zones 2 and 5, respectively). In these regions, the produced CH₄ is susceptible to oxidation by methanotrophic microbes before being released to the atmosphere, resulting in a rapid reduction of CH₄ emission (Figure S7 in Supporting Information S1). Optimizing the amount of GM biomass incorporated could be one of the essential solutions to reduce CH₄ emission in hotspots, particularly when considering the long-term impact in the assessment. We evaluated the annual GM production rate at regional scale (Figure S8 in Supporting Information S1), but lacked detailed information on the amount of biomass incorporated. This prevented us from conducting an in-depth analysis of CH₄ emission variations based on GM biomass input rates, as well as due to the common practice of incorporating the entire GM biomass into soils, which hinders a finer manipulation of GM application rates.

Milk vetch is the most representative GM crop in southern China, accounting for more than 80% of GM crops (Gao et al., 2023). As a leguminous winter crop commonly grown in paddy fields, it reduces synthetic N fertilizer input through atmospheric N₂ fixation. Compared to other GM crops such as gramineous (e.g., ryegrass) and cruciferous (e.g., rape) species, milk vetch exhibits lower biomass, which contributes to its observed CH₄ emission reduction effects in southern China. In addition to the GM rotation year and application rate, the type of GM crop can potentially affect CH₄ effects, which are regulated by the C/N ratio of the GM biomass. Long-term GM rotation with a low C/N ratio, such as milk vetch and morning glory, which have a ratio of approximately 11, can reduce annual CH₄ emissions by 16.3%–25.3%. In contrast, GM with a high C/N ratio, such as ryegrass, rape, and dhaincha, with a ratio of approximately 25, can increase CH₄ emissions by 21.7%–116.7% (Datta et al., 2013; Raheem et al., 2019). Incorporating GM with a low C/N ratio to regulate the high C/N ratio of rice straw can effectively reduce CH₄ emissions in paddy field systems (Zhou et al., 2020). Our research findings indicate that climate and soil conditions primarily influence CH₄ emissions indirectly by affecting the biomass production of GM crops. While milk vetch's CH₄ mitigation effects are well-demonstrated in southern China, its application in other rice-growing regions requires careful consideration of local conditions and GM characteristics, particularly biomass and C/N ratio. Although this study evaluates the most representative GM crop in terms of CH₄ effects, future research incorporating a wider variety of GM crops would enable more accurate quantification of these effects at broader regional or global scales.

Our estimates are subject to several sources of uncertainties. On the one hand, additional mitigation strategies beyond GM application rate are not well considered in this study, such as optimized irrigation management (Hou et al., 2022; Tariq et al., 2017) or GM crop mixtures (Hwang et al., 2017). Recent studies reveal that delayed flooding following GM incorporation promotes aerobic decomposition of organic materials during the initial phase, thereby reducing DOC concentrations in the rice growing season. This process decreases substrate availability for methanogens and significantly mitigates CH₄ emissions (Ma et al., 2024). Integrating such strategies into predictive models could substantially shorten the 15-year mitigation timeline for CH₄ reduction. On the other hand, the potential for CH₄ production and oxidation is characterized by the ratios of *mcrA/pmoA* (or *mcrA/(pmoA + NC10 + SR)*) gene abundances (Zhou et al., 2020). However, the biological and abiotic mechanisms driving changes in methane-oxidizing microbes within the GM-rice system remain unclear, particularly regarding the impacts of interactions between GM plant roots and soil. Studies have shown that during GM growth, root exudates can enhance particulate organic carbon fractions in soil through increased granular cementation while reducing microbial assimilation of organic carbon (Oleghe et al., 2019); On the other hand, root exudates also mediate CH₄ production and oxidation processes by altering soil pH and redox potential (Girkin et al., 2018). Future work should focus on conducting controlled experiments to provide more direct evidence for the time-dependent relationship between GM rotation years and CH₄ emissions, while addressing key uncertainties such as limited data on GM biomass input rates. A more comprehensive and policy-relevant GM management framework should be developed, incorporating optimized GM biomass along with efficient irrigation and drainage schemes, while explicitly evaluating the implications of GM biomass variability. Ultimately, our study provides substantial scientific insights for assessing the environmental consequences of other agro-economic practices, beyond GM application, but underscores the need for long-term mechanistic research to guide sustainable policies.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

The executable WHCNS model and code for regional modeling are available in Liang (2024a). The data set of observed CH₄ emission fluxes from experimental sites for model calibration and validation is available in Liang (2024b). Gridded spatial distributions of GM biomass in southern China is available in Liang (2025).

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