

Phosphorus makes the difference: N/P stoichiometry governs greenhouse gas emissions in manure-amended black soils

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Research Article

Keywords: manure substitution, soil N/P ratio, greenhouse gas emissions, phosphorus limitation, black soil

Posted Date: September 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10886348/v1>

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Additional Declarations: No competing interests reported.

Abstract

Purpose

Long-term intensive tillage and excessive chemical fertilizer have caused severe greenhouse gas (GHG) emissions from Northeast China's black soil. Manure substitution is promising but controversial, and quantitative evidence linking soil C/N/P stoichiometry to GHG emissions remains lacking. This study evaluated manure substitution effectiveness in restoring stoichiometric balance and mitigating GHG emissions.

Methods

A three-year field experiment compared manure substitution (20% organic nitrogen) under no-tillage (NTM) and conventional tillage (CTM) against chemical fertilizer alone (NTC and CTC), to evaluate its effectiveness in restoring soil C/N/P stoichiometric balance and mitigating GHG emissions.

Results

NTM significantly increased soil organic carbon (SOC) and total nitrogen (TN) contents and enhanced CH_4 uptake by 24.3%-62.4%, but accelerated CO_2 and N_2O emissions by 22.0%-57.2% and 5.7%-88.6%, respectively, raising global warming potential (GWP) by 21.7%-57.8%. Multivariate analyses consistently identified the N/P ratio as the primary predictor of GHG emissions. Manure substitution elevated soil N/P ratios (2.80–4.14) and C/P ratios (27.99–40.52) above regional averages (2.03 and 22.82, respectively), indicating a stoichiometric imbalance with C/N enrichment and phosphorus (P) limitation. This imbalance was further amplified by manure substitution, increasing CO_2 and N_2O emissions, while SOC and TN were significantly correlated only with C/P and N/P ratios, confirming P status as the dominant regulator of C/N turnover.

Conclusion

Manure substitution enhanced soil C and N pools but exacerbated GHG emissions by altering stoichiometric balance, with N/P ratio as a key design parameter for balancing carbon sequestration and GHG mitigation in degraded black soil restoration.

1. Introduction

Northeast China harbors one of the world's three largest black soil regions, a critical agroecosystem that has sustained grain production for decades (Gong et al. 2024). However, long-term high-intensity utilization and intensive agricultural management, especially the excessive application of chemical fertilizers, have led to a continuous decline in black soil organic matter and an imbalance in soil nutrient

structure (Jiao et al. 2025). This degradation not only weakens the carbon sequestration potential of black soil but also significantly induces intense GHG emissions. Due to its vast cultivation area, upland black soil serves as a major contributor to regional atmospheric GHG emissions (Wang et al. 2024b). Notably, studies show that well-aerated upland black soil is an important CH₄ sink, with an annual uptake of approximately 10–49 Tg, contributing 10% to the global CH₄ sink.

The excessive application of chemical fertilizers and soil degradation may weaken this critical sink function (Jiang et al. 2025), while GHG emissions caused by chemical fertilizer application account for 57% of total emissions from agricultural inputs in China (Cheng et al. 2011). Therefore, systematically monitoring and regulating soil nutrient status are key strategies to mitigate farmland GHG emissions and achieve sustainable agriculture.

In agroecosystems, manure application is an effective approach to enhance soil fertility; consequently, the total area of manure application in China reached approximately 24.67 million hectare-times (370 million mu-times) in 2023 (Zhang 2025). However, the effects of manure application on soil GHG emissions are controversial (Hu et al. 2023, Wu et al. 2024). Some studies suggest that manure can inhibit GHG emissions by reducing chemical fertilizer reliance and promoting carbon-nitrogen coupling (Xu et al. 2024, Zou et al. 2025), whereas other studies find that manure application leads to a surge in N₂O emissions (Shakoor et al. 2021, Zhou et al. 2017). This difference in effects primarily depends on changes in soil stoichiometric ratios. Manure application not only increases the availability of labile nitrogen but also regulates microbial metabolic activity by changing the supply-demand balance of soil C, N, and P, thus becoming a core factor driving GHG emission fluctuations (Arenberg & Arai 2019, Guenet et al. 2021).

Soil C, N, and P govern nutrient cycling and trace gas exchange in agroecosystems, while their stoichiometric ratios couple external nutrient inputs, microbial activity, and ecosystem functions (Hu et al. 2022, Song et al. 2024). Existing studies have shown that management practices such as fertilization and tillage can significantly change soil C/N/P ratios, driving microbes to shift their metabolic strategies and thereby regulating GHG emission pathways (Zhu et al. 2018). For example, imbalances in C/P or N/P ratios can induce microbial nutrient compensation strategies to acquire limited nutrients, and this priming effect often accelerates the decomposition of stable SOC, leading to increased CO₂ emissions (Zhu et al. 2018). Conversely, an increase in the C/N ratio can enhance the microbial immobilization of inorganic nitrogen, effectively reducing denitrification substrates and thus inhibiting N₂O production (Alam et al. 2023). Compared to other ecosystems (Wang et al. 2024a, Wang et al. 2017), the C/N/P ratios in black soil farmlands are more strongly disturbed by human activities (Li et al. 2025), and the shifts in these stoichiometric characteristics play a regulatory role in driving GHG emissions in black soil.

The study aims to address the following scientific questions: (1) To quantify the changes in soil C, N, P contents and their stoichiometric ratios under combined manure and straw incorporation over three years. (2) To evaluate the effects of manure on soil GHG emissions and global warming potential (GWP)

under different tillage practices in maize planting system. (3) To elucidate the critical pathways through which soil stoichiometric ratios mechanistically drive GHG emissions. The results of this study will provide a theoretical basis for achieving green agricultural development, optimizing nutrient management, and formulating climate change mitigation strategies in China's black soil region.

2. Materials and methods

2.1. Experimental site

The study was conducted at the Changchun Comprehensive Agricultural Experiment Station of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences (2023–2025). The research site is located in a typical black soil region of the central Songliao Plain (44°00'N, 125°24'E), characterized by a temperate semi-humid continental monsoon climate with large diurnal temperature fluctuations and warm, rainy summers. The multi-year average temperature is approximately 6.4°C, and the annual precipitation is about 614 mm, occurring primarily from July to September. The soil at the site is classified as typical black soil (Mollisol), derived from Quaternary loess-like sediments, with a predominantly loamy texture.

2.2. Experimental design

Since 2012, the experimental plots have been under a continuous maize planting system with full straw return. The manure application experiment was initiated in 2023. Maize is typically sown in early May and harvested in early to mid-October each year.

The experiment followed a two-factor randomized complete block design with four treatments and four replicates per treatment. The two factors were tillage mode (CT: conventional tillage; NT: no-tillage) and fertilization strategies (C: 100% chemical fertilizer; M: 20% organic manure nitrogen substituting for chemical nitrogen) including NTM (NT with 20% organic manure nitrogen substituting chemical nitrogen), NTC (NT with 100% chemical fertilizer), CTM (CT with 20% organic manure nitrogen substituting chemical nitrogen), and CTC (CT with 100% chemical fertilizer). All treatments were performed under equal nitrogen (N) and full straw return conditions (approximately 10,000–11,000 kg·hm⁻²). The four treatment combinations were as follows (Table 1).

Table 1
Nitrogen application rates, proportions of manure and chemical fertilizer nitrogen, and straw management across different tillage methods

Treatment	Tillage method	Total nitrogen application rate (kg ha ⁻¹)	Proportion of manure nitrogen (%)	Proportion of Chemical Fertilizer Nitrogen (%)	Straw Management
NTM	no-tillage	130	20	80	Full-straw returning
NTC	no-tillage	130	0	100	Full-straw returning
CTM	conventional tillage	130	20	80	Full-straw returning
CTC	conventional tillage	130	0	100	Full-straw returning

Following the annual autumn harvest, maize straw was pulverized and fully returned to the soil, with about 10,000–11,000 kg·ha⁻². In CT, the straw and stubble residues were incorporated into the 0–20 cm soil layer via moldboard plowing after harvest; whereas under NT, residues remained undisturbed on the soil surface. Basal fertilizers were applied as a single dose during spring sowing via surface broadcasting. The chemical fertilizer contained 25% N, 10% P₂O₅, and 16% K₂O. The organic manure was prepared through thermophilic composting of livestock manure and maize straw. The key physicochemical properties of the manure, including total nitrogen and water content, during 2023–2025 are detailed in Table S1.

2.3. Sample collection and measurement

2.3.1. Soil sampling and analytical methods

Topsoil samples (0–20 cm) were collected from each plot during the sowing, tasseling, grain-filling, and harvest stages of maize. In each plot, three soil cores were randomly collected and pooled to form a single composite sample. After removing visible plant residues, stones, and other debris, the soil samples were air-dried at room temperature. The dried soil was then crushed and passed through a 2-mm sieve to determine soil pH and electrical conductivity (EC). A representative portion of the screened soil was further ground to pass through a 0.149-mm sieve (100-mesh) to analyze the concentrations of soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP).

Soil pH was measured in a 5:1 (w/v) water-to-soil suspension using a portable pH meter (SX723, Shanghai, China). Soil organic carbon (SOC) was determined by the potassium dichromate oxidation-sulfuric acid titration method. Total nitrogen (TN) and total phosphorus (TP) were determined using an

automated discrete analyzer (Smartchem 200, Italy) after digestion with sulfuric acid-salicylic acid and sulfuric acid-perchloric acid, respectively (Song et al. 2025).

2.3.2. Greenhouse gas (GHG) collection and measurement

Soil CO₂, CH₄, and N₂O fluxes were measured using the static opaque chamber-gas chromatography method. Stainless steel chamber bases were permanently installed in each plot. During the maize growing seasons (2023–2025), gas samples were collected between 09:00 and 11:00 to represent the daily mean flux. Sampling frequency was adjusted based on the growth stage, twice within the first week after sowing, weekly during the first month, and once every 2 weeks until harvest. At each sampling event, gas samples were collected from the headspace at 0, 10, 20, and 30 min using 30 mL vacuum syringes. Gas concentrations were determined using a gas chromatograph (7890B, Agilent, USA) equipped with an electron capture detector (ECD) and a flame ionization detector (FID). GHG fluxes (F), cumulative emissions (E), and the area-scaled global warming potential (GWP) were calculated following the equations described by Wang et al. (2022):

$$F = \rho \times h \times \frac{dc}{dt} \times \frac{273}{273+t} \quad \# (1)$$

where F is the emission flux of CO₂, CH₄, or N₂O (μg m⁻² h⁻¹ or mg m⁻² h⁻¹); ρ is the gas density at standard conditions (CO₂ = 1.964 kg·m⁻³, CH₄ = 0.714 kg·m⁻³, N₂O = 1.964 kg·m⁻³); h is the chamber height (m); dc/dt is the rate of change in the concentration of CO₂, CH₄, or N₂O inside the chamber; and t is the mean air temperature inside the chamber during the four sampling times (°C).

Cumulative GHG emissions were calculated using the following formula:

$$E = \sum_{i=1}^n \frac{F_i + F_{i+1}}{2} \times (t_{i+1} - t_i) \times 24 \quad \# (2)$$

where F and E are the emission flux and cumulative emission of CO₂, CH₄, or N₂O (kg ha⁻¹ or Mg ha⁻¹), n is the total number of gas sampling events, i is the i -th gas sampling event, and $t_{i+1} - t_i$ is the time interval between two adjacent sampling events.

The global warming potential (GWP, kg CO₂-eq ha⁻¹) of soil GHGs in upland black soil was calculated using the following formula (Wang et al. 2022):

$$GWP = E(\text{CO}_2) + 25 \times E(\text{CH}_4) + 298 \times E(\text{N}_2\text{O}) \quad \# (3)$$

where GWP is the global warming potential, and $E(\text{CO}_2)$, $E(\text{CH}_4)$, and $E(\text{N}_2\text{O})$ are the cumulative emissions of CO₂, CH₄, and N₂O, respectively. The coefficients 298 and 25 are the GWP factors (kg CO₂-eq ha⁻¹) used to convert N₂O and CH₄, respectively, into CO₂ equivalents.

2.4. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA). A two-way repeated measures ANOVA was conducted to evaluate the effects of treatment, year, and their

interactions on GHG emissions and soil properties, followed by Tukey's HSD test for post-hoc multiple comparisons ($p < 0.05$). The remaining statistical analyses were conducted in RStudio; the Mantel test and Redundancy Analysis (RDA) were employed to explore the relationships between GHG emissions and soil physicochemical variables (He et al. 2026). To address multicollinearity, variables with a variance inflation factor (VIF) > 10 were excluded, and forward selection was performed using the `ordiR2step` function to optimize the model. Furthermore, Random Forest regression was applied to quantify the relative importance of environmental drivers for GHG emissions (He et al. 2023). All figures were generated using Origin 2026 and the `ggplot2` package in RStudio.

3. Results

3.1. Effects of manure substitution on soil nutrient content

3.1.1. SOC, TN, and TP Content

Both fertilization treatments, experimental years, and their interactions exerted significant effects on SOC, TN, and TP contents (Table S2, Fig. 1). Fertilization practices significantly influenced soil nutrient dynamics, with manure substitution (NTM and CTM) showing a superior capacity for nutrient sequestration compared to chemical fertilization alone (NTC and CTC), particularly under the NT system. In the NT system, the superiority of manure over chemical fertilizer became increasingly evident from 2023 to 2025. Specifically, the SOC growth increment in the NTM treatment (1.33 g/kg) exceeded that in the NTC treatment (1.24 g/kg), resulting in a cumulative growth rate of 6.65% by 2025. The response of TN was even more substantial; the NTM treatment achieved a growth rate of 25.96% (0.43 g/kg), outperforming the 21.22% growth (an increment of 0.40 g/kg) observed in the NTC treatment. While TP content remained relatively stable, manure substitution (NTM and CTM) maintained positive growth rates of 2.72%, whereas the NTC treatment exhibited a slight decline of 1.33%. These results show that the NTM model is the most effective strategy for long-term fertility enhancement, with its carbon and nitrogen sequestration benefits increasing as cultivation duration increases.

3.1.2. SOC, TN, and TP stoichiometry

The monitoring results from 2023 to 2025 showed that the soil stoichiometric ratios, including C/N, C/P, N/P, and C/N/P, exhibited clear inter-annual dynamics and differences among treatments (Table S2; Fig. 2).

The soil C/N ratio declined significantly across all treatments from 2023 to 2025 (Fig. 2a). In the NT system, the soil C/N ratio in the NTM treatment remained consistently and significantly higher than that in the NTC treatment throughout the experimental period, despite decreasing from 12.15 in 2023 to 10.53 in 2025, compared to the decline from 10.33 to 9.02 observed in the NTC treatment. A similar trend was observed in the CT system, although at a lower baseline level. The CTM treatment (decreased by 11.69%) consistently outperformed the CTC treatment (decreased by 8.57%).

The soil C/P ratio varied among treatments and fluctuated from 2023 to 2025 (Fig. 2b). Within the NT system, the NTM treatment reached a statistically significant peak of 39.41 in 2024, with its cumulative three-year increase (9.05%) being significantly higher than that observed in the NTC treatment (5.99%). Notably, in the CT system, although no significant difference was observed between CTM and CTC in 2023, the C/P ratio in the CTM treatment (31.54) significantly exceeded that in the CTC treatment (30.25) by 2025, as the experiment progressed.

The soil N/P ratio increased significantly across all treatments from 2023 to 2025 (Fig. 2c). In the NT system, both the NTM and NTC treatments exhibited an upward trend; the N/P ratio in the NTM treatment increased from 3.16 in 2023 to 3.98 in 2025, whereas that in the NTC treatment increased from 3.18 to 4.00. Notably, the superiority of manure substitution was more pronounced in the CT system; the CTM treatment achieved a remarkable increase from 2.89 to 3.53, significantly outperforming the CTC treatment, which increased from 3.03 to 3.51.

The soil C/N/P molar ratio exhibited divergent dynamic trends across all treatments from 2023 to 2025. In the NT system, the NTM treatment reached a peak C/N/P ratio of 101.57:7.61:1 in 2024 before stabilizing at 99.63:8.82:1 in 2025, which remained consistently higher than the ratio of 90.23:8.86:1 observed in the NTC treatment. A similar enhancement was observed in the CT system, although at a lower baseline stoichiometry. Despite the CTM treatment (75.67:6.38:1) initially having a lower C/N/P ratio than the CTC treatment (76.91:6.69:1) in 2023, the CTM treatment achieved a more robust stoichiometric improvement by 2025 (81.30:7.83:1), outperforming the CTC treatment (77.96:7.78:1).

3.2. Effects of manure substitution on soil GHG emissions

3.2.1. CO₂ emissions

Soil CO₂ emission fluxes exhibited significant seasonal fluctuations during the maize growing season, ranging from 82.89 to 1220 mg·m⁻²·h⁻¹ (Fig. 3a). Over the three-year monitoring period, manure substitution significantly increased three-year total cumulative CO₂ emissions (Fig. 3b-1), with this stimulatory effect being more pronounced under the NT system. Specifically, the NTM treatment led to a three-year total cumulative CO₂ emissions as high as 72.50 Mg ha⁻¹, representing a 57.2% increase compared to the NTC treatment (46.11 Mg ha⁻¹); meanwhile, CTM (71.58 Mg ha⁻¹) also showed a 22.0% increase compared to CTC (58.65 Mg ha⁻¹). Annual dynamics (Fig. 3b) showed that the stimulatory effect of manure substitution on CO₂ emissions was most pronounced in 2023, with the CTM treatment increasing CO₂ emissions by 44.79% compared to CTC. As the experimental duration extended, a substantial reduction in soil CO₂ emissions was observed across all treatments. By 2025, cumulative emissions had decreased by 26.15% to 42.12% relative to those in 2023.

3.2.2. CH₄ emissions

Soil CH₄ fluxes exhibited alternating patterns of emission and uptake during the maize growing season, ranging from - 89.79 to 90.56 µg·m⁻²·h⁻¹ (Fig. 3c). Typically, CH₄ uptake intensity weakened or showed

brief emission peaks following chemical fertilizer application. Over the three-year monitoring period, manure substitution significantly enhanced three-year total cumulative CH_4 sink intensity (Fig. 3d-1), with this effect being more pronounced under the NT system. Specifically, the NTM treatment led to a three-year total cumulative CH_4 uptake of $-2.39 \text{ kg}\cdot\text{ha}^{-1}$, representing a 62.4% increase in sink strength compared to the NTC treatment ($-1.47 \text{ kg}\cdot\text{ha}^{-1}$). Similarly, under the CT system, the CTM treatment ($-1.50 \text{ kg}\cdot\text{ha}^{-1}$) exhibited a 24.3% increase in CH_4 uptake compared to the CTC treatment ($-1.20 \text{ kg}\cdot\text{ha}^{-1}$). Annual dynamics (Fig. 3d) further showed that in addition to the exceptionally strong uptake in NTM during 2023 ($-1.12 \text{ kg}\cdot\text{ha}^{-1}$), the CTC treatment also exhibited a clear trend of enhanced uptake in 2025.

3.2.3. N_2O emissions

Similar to CO_2 trends, fertilization was the primary driver of soil N_2O emission peaks (Fig. 3e). Over the three-year observation period, N_2O fluxes increased rapidly following fertilization and exhibited intense fluctuations, ranging from -19.85 to $254.60 \mu\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, during which manure substitution significantly increased cumulative N_2O emissions across both NT and CT systems. Further analysis of the cumulative emissions (Fig. 3f-1), revealed that this stimulatory effect was substantially more pronounced under the NT system. Specifically, the NTM treatment led to a three-year total cumulative N_2O emission of up to $5.57 \text{ kg}\cdot\text{ha}^{-1}$, representing an 88.6% increase compared to the NTC treatment ($2.95 \text{ kg}\cdot\text{ha}^{-1}$). In contrast, under the CT system, although the CTM treatment recorded the highest three-year total cumulative emissions ($5.94 \text{ kg}\cdot\text{ha}^{-1}$), it showed a relatively smaller increase of 5.7% compared to the CTC treatment ($5.62 \text{ kg}\cdot\text{ha}^{-1}$). Annual dynamics (Fig. 3f) showed that the stimulatory effect of manure substitution on N_2O emissions was initially most prominent under the CT system in 2023, with the CTM treatment increasing N_2O emissions by 108.3% compared to CTC. As the experimental duration extended, this stimulatory effect intensified under the NT system. By 2025, the N_2O emissions of the NTM treatment were 153.9% higher than those of the NTC treatment ($0.68 \text{ kg}\cdot\text{ha}^{-1}$).

3.3. Global warming potential

Two-way ANOVA indicated that the experimental year, fertilization treatment, and their interaction significantly influenced the GWPs of individual gases (CH_4 and N_2O) and the total GWP ($p < 0.01$). Throughout the three-year monitoring period, the total GWP fluctuated between 12,472 and 32,476 $\text{kg CO}_2\text{-eq ha}^{-1}$. The highest annual total GWP was recorded in the CTM treatment in 2023, while the NTC treatment consistently maintained relatively low levels throughout the study period. Manure substitution significantly increased the GWP under both the NT and CT systems ($p < 0.05$). Specifically, under the NT system, the cumulative GWP of the NTM treatment was $74,101 \text{ kg CO}_2\text{-eq ha}^{-1}$, representing a significant increase of 57.8% compared to the NTC treatment ($46,951 \text{ kg CO}_2\text{-eq ha}^{-1}$). Similarly, under the CT system, the cumulative GWP of the CTM treatment reached $73,312 \text{ kg CO}_2\text{-eq ha}^{-1}$, which was 21.7% higher than that of the CTC treatment ($60,230 \text{ kg CO}_2\text{-eq ha}^{-1}$).

Table 2

Two-way ANOVA of the warming potential of individual gases and total GWP in response to year and treatment in maize planting system

Year	Treatment	Global warming potential (kg CO ₂ -eq ha ⁻¹)			Total GWP (kg CO ₂ -eq ha ⁻¹)
		CO ₂	CH ₄	N ₂ O	
2023	NTM	28176 ± 2299 ab	-27.96 ± 2.41 c	596.57 ± 116.46 b	28744 ± 2417 ab
	NTC	18890 ± 2184 c	-13.33 ± 0.76 b	410.74 ± 161.49 c	19288 ± 2346 c
	CTM	31726 ± 2089 a	-19.15 ± 0.78 b	769.13 ± 334.11 a	32476 ± 2424 a
	CTC	21912 ± 2201 bc	-6.68 ± 2.29 a	354.61 ± 92.80 c	22259 ± 2296 bc
2024	NTM	27490 ± 2150 a	-25.08 ± 0.39 c	545.29 ± 120.43 a	28011 ± 2271 a
	NTC	14941 ± 1783 b	-11.21 ± 2.30 bc	265.29 ± 16.70 b	15195 ± 1802 b
	CTM	21488 ± 4052 ab	-10.52 ± 0.88 b	293.22 ± 64.91 b	21771 ± 4117 ab
	CTC	20558 ± 1708 ab	-9.54 ± 0.77 a	527.21 ± 47.79 a	21075 ± 1757 ab
2025	NTM	16836 ± 1802 ab	-6.67 ± 1.98 a	516.55 ± 284.39 bc	17345 ± 2088 ab
	NTC	12276 ± 1619 b	-12.21 ± 0.22 c	203.49 ± 56.79 c	12468 ± 1676 b
	CTM	18364 ± 1487 a	-7.72 ± 0.49 b	708.08 ± 360.96 ab	19064 ± 1848 a
	CTC	16182 ± 1503 ab	-13.88 ± 1.55 d	726.08 ± 145.28 a	16894 ± 1650 ab
ANOVA	Year	**	**	**	**
	Treatment	**	**	**	**
	Year × Treatment	NS	**	**	*

Note: Different lowercase letters indicate significant differences among treatments within the same season ($p < 0.05$). NS, *, and ** denote non-significance, $p < 0.05$, and $p < 0.01$, respectively.

3.4. Relationships between GHG emissions, GWP, soil physicochemical properties, and nutrient stoichiometric ratios

The interrelationships between soil physicochemical properties, nutrient stoichiometric ratios, and GHG emissions were comprehensively evaluated using Spearman correlation analysis and Mantel tests (Fig. 4). Mantel tests revealed that CO₂ emissions were significantly associated with SOC, TN, TP, C/N, N/P, and the C/N/P ratio, exhibiting significant negative correlations with SOC, TN, TP, and the N/P ratio, while showing significant positive correlations with the C/N and C/N/P ratios. Soil CH₄ intensity was significantly and positively correlated with pH, C/N, and the C/N/P ratio. N₂O emissions displayed strong positive correlations with the C/N and C/N/P ratios, but were negatively correlated with the N/P ratio and TP. Furthermore, the primary drivers of GWP were identified, demonstrating that GWP was positively correlated with the C/N/P ratio, C/N ratio, and EC, while showing significant negative associations with SOC, TN, TP, and the N/P ratio.

Redundancy analysis (RDA) further elucidated the influence of soil physicochemical properties and nutrient stoichiometric ratios on GHG emissions within a multidimensional space. The full-factor RDA (Fig. 5a) showed that the first two axes (RDA1 and RDA2) accounted for 75.16% and 15.53% of the total variance, respectively. Spatially, treatment clusters under chemical fertilization alone (NTC and CTC) were closely associated with high baseline nutrient indicators, particularly TP, TN, and the N/P ratio. Conversely, manure substitution treatments (NTM and CTM) exhibited a distinct shift toward the C/N/P and GWP vector, highlighting a strong coupling between manure application and intensified GHG emissions. Monte Carlo permutation tests (Fig. 5b) revealed that N/P, C/N/P, TP, C/N, SOC, and TN were significant predictors of GHG emissions, with the N/P ratio exhibiting the highest explanatory contribution. Through a parsimonious model refined by excluding redundant variables (Fig. 5c, d), the first two axes (RDA1 and RDA2) explained 85.93% and 13.86% of the total variance, respectively, highlighting that CO₂ and N₂O fluxes, as well as the total GWP, were strongly and positively correlated with soil C/N/P ratio, while the soil N/P ratio exerted a significant negative influence on GWP variation. Additionally, CH₄ uptake intensity showed a significant positive association with the N/P ratio, such that an organic-induced reduction in the soil N/P ratio shifts the net CH₄ balance toward enhanced uptake.

To identify the key drivers of GHG emissions, a random forest (RF) model was employed to quantify the relative importance of each predictor variable (Fig. 6). The results revealed that the soil N/P ratio emerged as the most critical predictor of CO₂ and N₂O emissions, with variable importance scores of 14.5% and 16.1% increase in the mean squared error (%IncMSE), respectively (Fig. 6a, c). A comprehensive analysis of GWP indicated that the soil N/P ratio exerted the strongest predictive influence (14.2% IncMSE), followed by C/N/P, SOC, and the cumulative CO₂ and CH₄ fluxes, all of which were significant predictors of GWP (Fig. 6d).

4. Discussion

4.1. Effects of partial manure substitution on soil nutrient stoichiometric balance

Compared with the chemical fertilizer alone treatments, the manure substitution treatments exhibited stronger C and N sequestration capacity (Fig. 1), which primarily originated from the direct input of exogenous C providing a carbon source foundation for the soil, while aggregate physical protection and microbial stabilization further hindered the rapid decomposition of organic matter (Xu et al. 2023); at the same time, manure substitution limited the immediate availability of nutrients by slowing down mineralization rates and microbial immobilization, thereby effectively avoiding stoichiometric nutrient surplus (Wang et al. 2025).

In this study, the C/N ratio in manure substitution treatments remained consistently higher than that under chemical fertilization alone. Over the three-year experimental period, the C/N ratios of all treatments showed a significant decreasing trend year by year (Fig. 2a). Most studies suggest that the soil C/N ratio is a key indicator for measuring organic matter turnover. When it falls below 16, it indicates that organic matter has been fully decomposed by microorganisms (Bui & Henderson 2013). In this study, the C/N ratios of all treatments were well below 16 (Fig. 2a), suggesting that the regional microenvironment favors microbial mineralization of organic matter and subsequent nutrient release (Baddeley et al. 2017). This phenomenon indicates that manure application significantly activated microbial metabolism and induced priming effects (Liu et al. 2025), which may have accelerated the mineralization and consumption of existing labile organic carbon, such that the net carbon accumulation rate failed to fully match the rapid nitrogen enrichment, ultimately leading to a sustained narrowing of the relative C/N ratio.

Unlike the C/N trend, the C/P ratio in manure substitution treatments was higher than that under chemical fertilizer alone during most periods in this study (Fig. 2b). Manure increases soil dissolved organic carbon, thereby raising the soil C/P ratio, and this change exacerbates microbial P limitation (Chen et al. 2026), which is consistent with the conclusions of Zhang et al. (2023). In this study, the low C/N ratio and high N/P ratio together indicated that N was relatively sufficient, while P limitation was prominent in the study area (Fig. 2). More notably, the soil N/P ratio in this study ranged from 2.80 to 4.14, which was significantly higher than the average level of Northeast upland soils (2.03) (Zhuo et al. 2019), but still lower than the national average (5.2) (Tian et al. 2010). This indicates that after manure input, the net accumulation rate of nitrogen has exceeded the effective supply rate of P. The input of organic manure led to a continuous increase in the N/P ratio (Fig. 2), promoting crop uptake of nutrients and water, which may become an important potential driving factor affecting regional GHG emission dynamics (Huang et al. 2023). Correlation analysis showed that SOC and TN contents were only significantly and positively correlated with the C/P and N/P ratios, but not with the C/N ratio (Fig. 4). This interesting result suggests that P is the key factor regulating the C and N cycling in upland black soil farmland. In other words, the accumulation behavior of C and N is not determined by their relative proportions, but rather depends more on the P supply status in the system. The underlying reason is that

P, as an important energy source for microbial metabolism, and its increased availability can significantly enhance microbial activity, thereby accelerating the turnover and release of active C and N components (Yu et al. 2025a, Zhang et al. 2022). Although no-tillage treatments achieved higher peak C and N enrichment in 2024 by reducing disturbance and strengthening physical protection, regardless of tillage practice, manure application significantly increased the intrinsic response induced by the N/P ratio compared with the chemical fertilization alone treatments. This stoichiometric imbalance may accelerate CO₂ release by promoting soil organic matter turnover, as similar findings have been reported in previous studies (Liu et al. 2025), but the underlying microbiological mechanisms remain to be further verified.

4.2. Soil Stoichiometric Characteristics Regulate GHG Emissions

Three-year monitoring revealed that compared with chemical fertilizer alone, manure substitution treatments significantly increased cumulative GHG emissions and GWP, while SOC, TN, and TP contents showed an increasing trend (Fig. 1; Fig. 3; Table 2). This indicates that organic matter input accelerated nutrient turnover and enhanced short-term GHG emissions in black soil. Therefore, from a short-term perspective, the NTC treatment performed better in balancing emission mitigation with fertility maintenance. However, manure addition also induced a significant upward trend in soil C/P and N/P ratios, indicating that this black soil system was gradually trending toward a P-limited state. Correlation analysis further confirmed that SOC and TN contents were significantly and positively correlated with C/P and N/P ratios (Fig. 4), corroborating that P is the key factor constraining C and N cycling in this upland soil system.

The aggravated P limitation induced an ecological stoichiometric compensation effect (Yu et al. 2025a, Zhang et al. 2022), whereby soil microorganisms were forced to enhance the priming and mineralization of organic matter in order to acquire scarce P sources. This microbial strategy accelerated the turnover of active C components and replenished substrates for heterotrophic respiration, directly leading to elevated CO₂ emissions under manure substitution treatments (Table 2; Fig. 3). In agreement with this, soil CO₂ flux was significantly negatively correlated with the N/P ratio and TP, but significantly positively correlated with the C/N ratio and C/N/P ratio (Figs. 4–6). While manure enriched the soil labile nutrient pool, the exogenous carbon input triggered a potent priming effect, accelerating the mineralization of existing SOC (Liu et al. 2025). RF models further confirmed that the predictive importance of the N/P ratio for CO₂ emissions far exceeded that of the C/N ratio (Fig. 6a), demonstrating that P-mediated priming effects are the core factor driving soil respiration intensification in upland black soil (Jílková et al. 2021).

Meanwhile, in this study, soil N₂O emissions showed a high positive correlation with the C/N and C/N/P ratios, and a significant negative correlation with the N/P ratio and TP (Fig. 4; Fig. 6c). On the one hand, the elevated N/P ratio inhibited the microbial assimilation capacity for inorganic N; under P limitation, microbial growth was restricted, and the fixation efficiency for excess N decreased, resulting in large

amounts of inorganic N remaining in the soil as substrates for nitrification and denitrification (Huang et al. 2020). On the other hand, the elevated C/N ratio provided sufficient energy for heterotrophic microorganisms, intensifying their respiratory oxygen consumption and creating local anaerobic conditions within microaggregates (Zhou et al. 2017). More critically, P deficiency may have inhibited the activity of N₂O reductase (NosZ) during denitrification (Cheng et al. 2025), and these mechanisms jointly caused large amounts of N₂O produced by denitrification to fail to be further reduced to N₂, ultimately manifesting as a surge in N₂O emissions. Mantel tests and RDA analyses consistently identified the soil N/P ratio as the environmental factor contributing the most to the explained variation in N₂O emissions (Fig. 5b; Fig. 5d), and the RF model also ranked its importance at 16.1% (Fig. 6c). In contrast, although manure substitution significantly enhanced CH₄ uptake capacity (Fig. 3), which may be attributed to increased methanotroph abundance and the alleviation of inorganic N inhibition on methane monooxygenase by organic matter (Meng et al. 2020), and CH₄ flux was positively correlated with the N/P ratio (Fig. 5), this positive contribution to the CH₄ sink was negligible in the overall GHG budget.

The soil N/P ratio was identified as a key factor influencing GHG emissions (Figs. 5–6). Comprehensive analysis showed that the soil N/P ratio had the highest predictive contribution to GWP (14.2%) (Fig. 6d). Although manure substitution treatments significantly improved CH₄ uptake capacity, the potent warming effects induced by CO₂ and N₂O emissions completely overwhelmed the minor contribution of enhanced CH₄ uptake, eventually leading to an increase in GWP. In summary, the soil N/P ratio is the core indicator regulating GHG emissions in upland black soil. Future research should further explore how the N/P ratio affects gas fluxes by mediating the metabolic trade-offs of microbial communities in microenvironments, providing a theoretical basis for the sustainable management of carbon sequestration and emission reduction in black soil.

5. Conclusions

Based on three consecutive years of field monitoring from 2023 to 2025, this study found that manure substitution, increased the contents of SOC, TN, and TP in the upland black soil of Northeast China, significantly. Specifically, the NTM treatment exhibited the most pronounced effect, increasing SOC, TN, and TP contents by 6.65%, 25.96%, and 2.72%, respectively. The addition of manure changed the soil C/N/P stoichiometric ratios, with the C/N, C/P, and N/P ratios shifting from 12.15, 36.14, and 3.16 in 2023 to 10.53, 31.54, and 3.98 in 2025, respectively. While enhancing soil fertility, manure substitution also increased CO₂ and N₂O emissions; particularly under the NTM treatment, it increased the three-year total cumulative CO₂ and N₂O emissions by 57.2% and 88.6%, respectively, ultimately leading to a significant 57.8% increase in the three-year total cumulative GWP. Soil C/N/P stoichiometric ratios were closely related to greenhouse gas emissions, and notably, the N/P ratio contributed the most to these emissions. Therefore, when promoting manure substitution strategies in the Northeast black soil region, it is necessary to weigh the benefits of carbon sequestration and fertility enhancement against the associated risks of warming. Future management should consider the soil N/P ratio as a core indicator to achieve sustainable agricultural production and carbon neutrality goals in the black soil region.

Declarations

Author Contributions

Lijuan Yang: Writing – original draft, Visualization, Validation, Software, Methodology, Data curation. Ping Li: Resources, Methodology, Investigation. Aizhen Liang: Writing – review & editing. Bing Bai: Conceptualization. Lixia Wang: Writing – review & editing, Supervision, Resources, Funding acquisition.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2023YFD1501103).

Data availability

Data will be made available on request.

Declarations of Competing Interests

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

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Figures

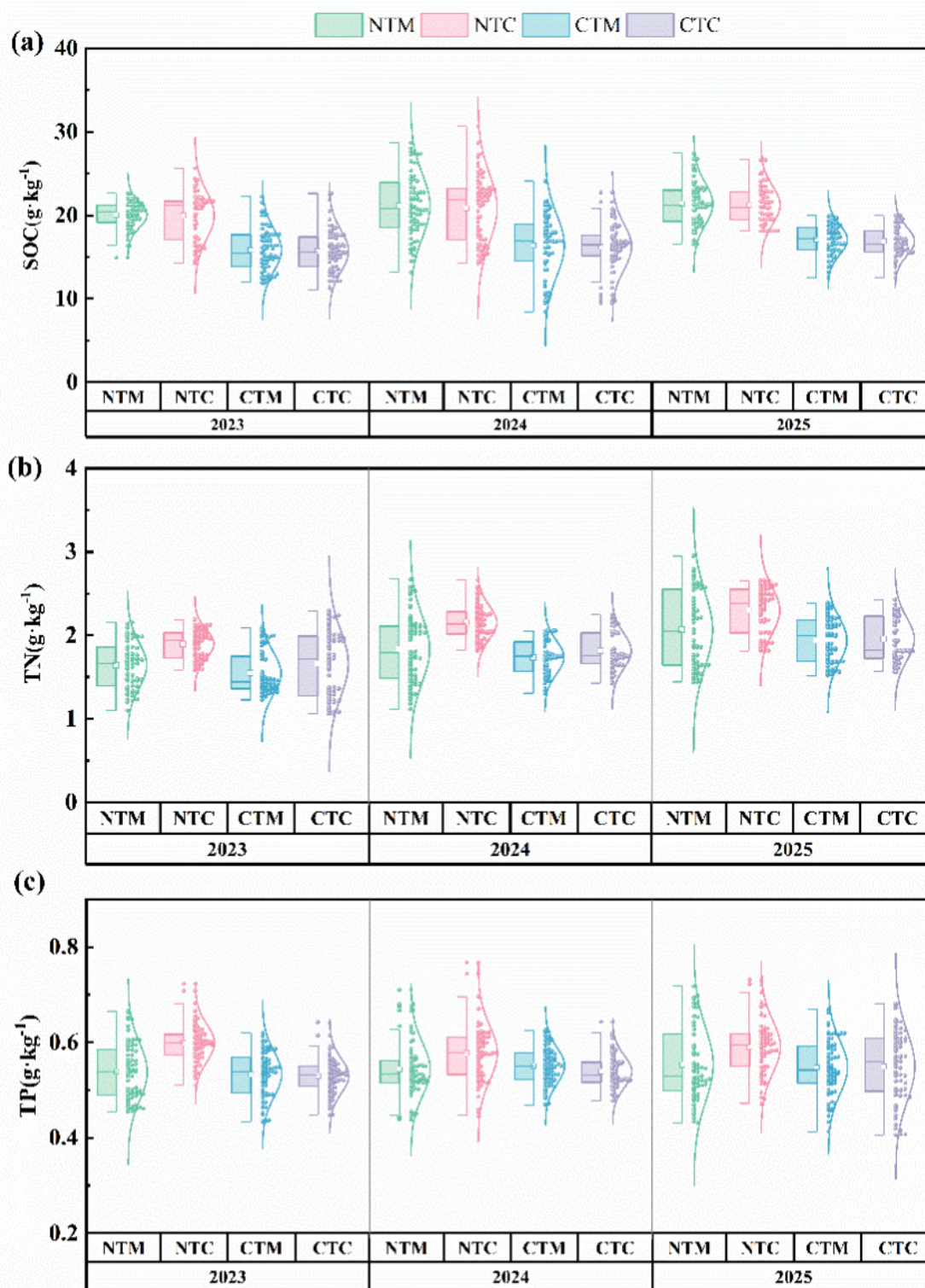


Figure 1

Dynamics of SOC, TN, and TP contents under different treatments from 2023 to 2025 NT: no-tillage; CT: conventional tillage; M: 20% substitution of chemical nitrogen with organic manure; C: 100% chemical fertilizer (same below). The left panel shows half-box plots. For each boxplot, the lower and upper boundaries of the box represent the 25th and 75th percentiles, respectively; the solid line and the

square marker inside the box indicate the median and the mean, respectively; the lower and upper whiskers show the minimum and maximum values, respectively

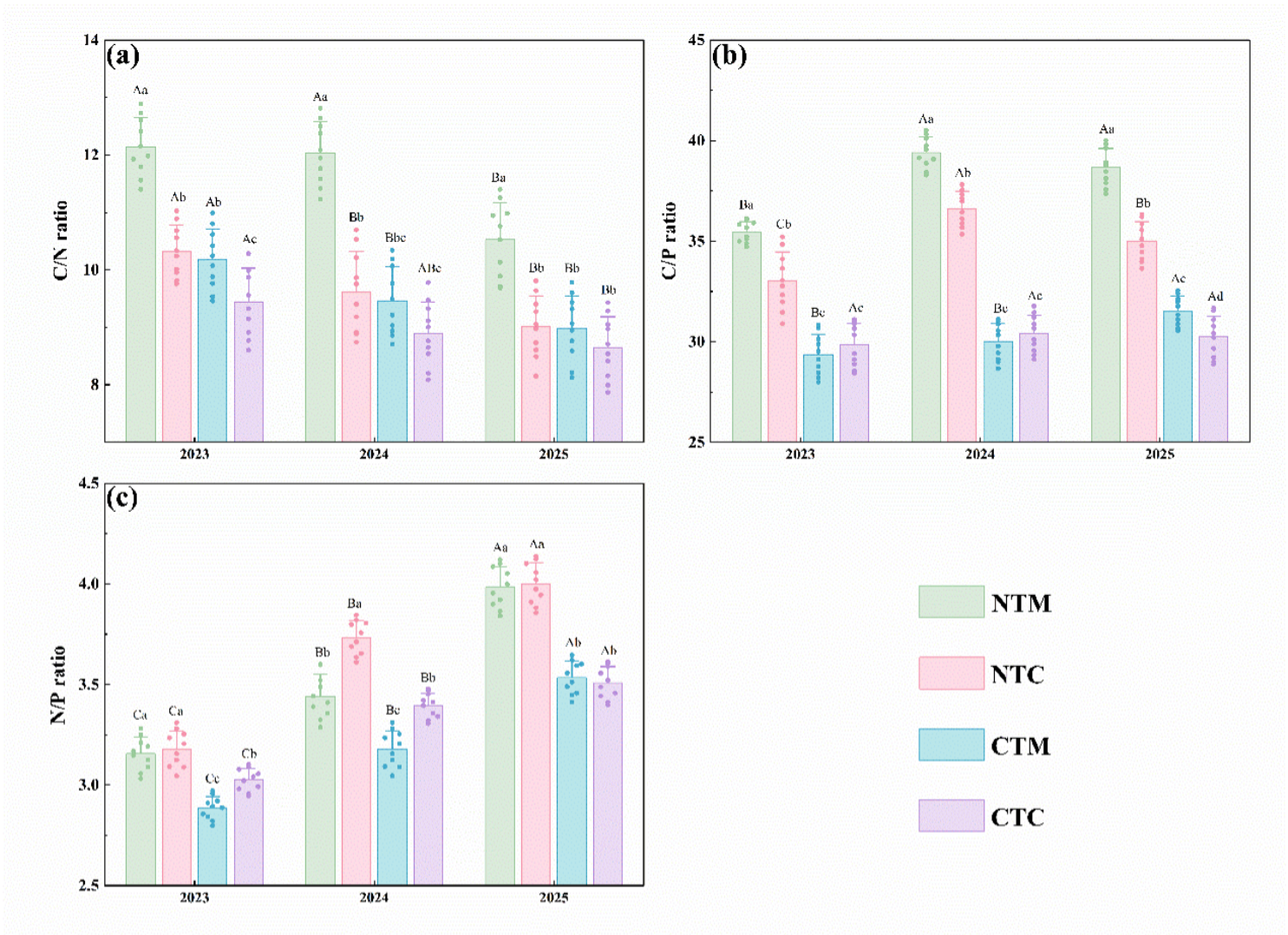


Figure 2

Changes over time in C/N, C/P, and N/P ratio under various treatments from 2023 to 2025 Data are presented as mean $\pm$ standard error. Lowercase letters above the bars show significant differences among treatments ($p < 0.05$), while uppercase letters indicate significant differences among years ($p < 0.05$). The same applies below

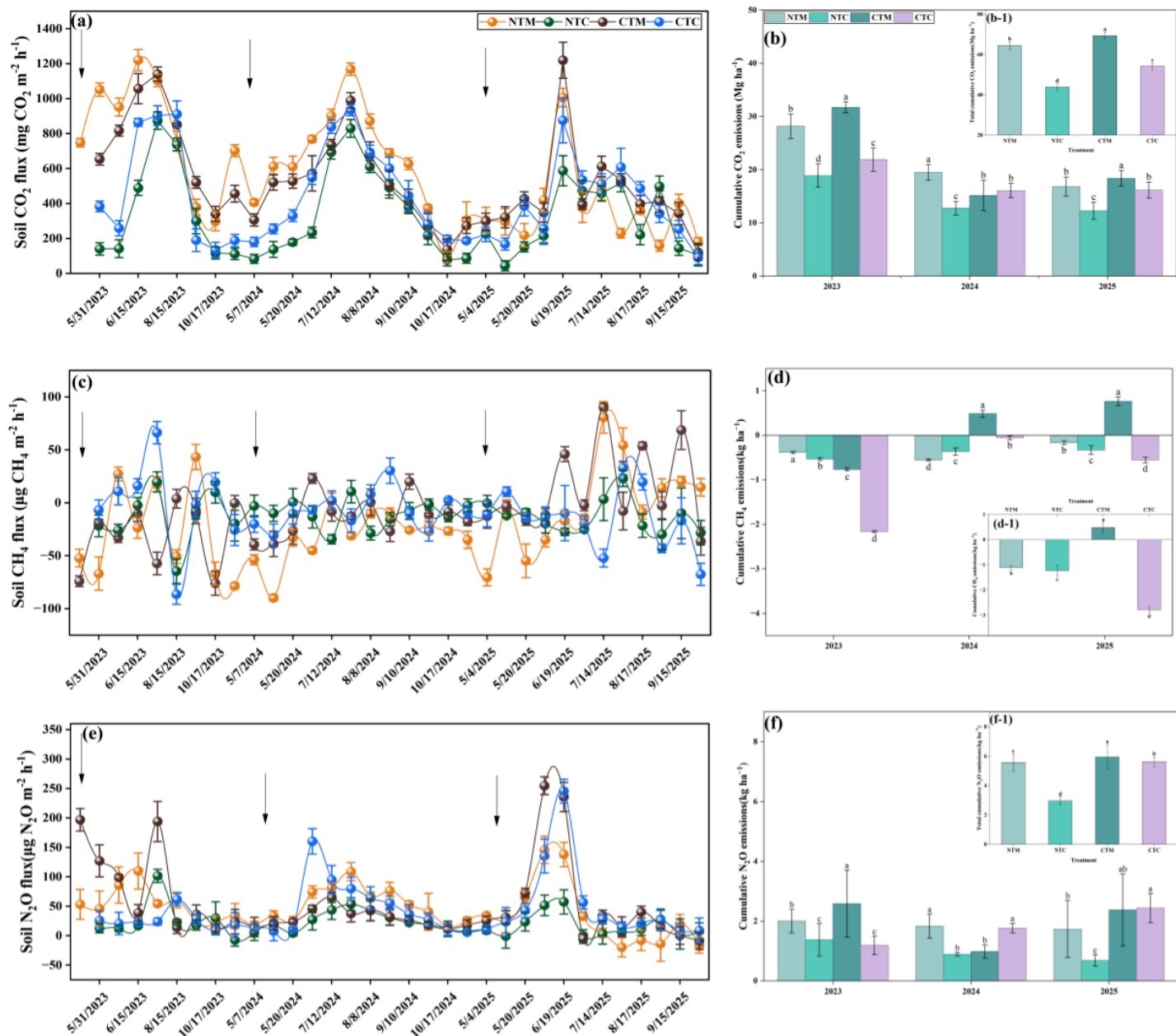


Figure 3

Effects of different treatments on soil GHG emission fluxes, cumulative emissions, and total cumulative emissions. (a), (c) and (e) represent the emission fluxes of soil CO₂, CH₄ and N₂O for 2023-2025, respectively; (b), (d) and (f) represent the cumulative emissions of soil CO₂, CH₄ and N₂O for 2023-2025, respectively; (b-1), (d-1) and (f-1) represent the total cumulative emissions of the respective gases over the three years. Data are shown as mean ± standard error (n = 3). Different lowercase letters above the bars indicate significant differences among treatments within the same period ($p < 0.05$)

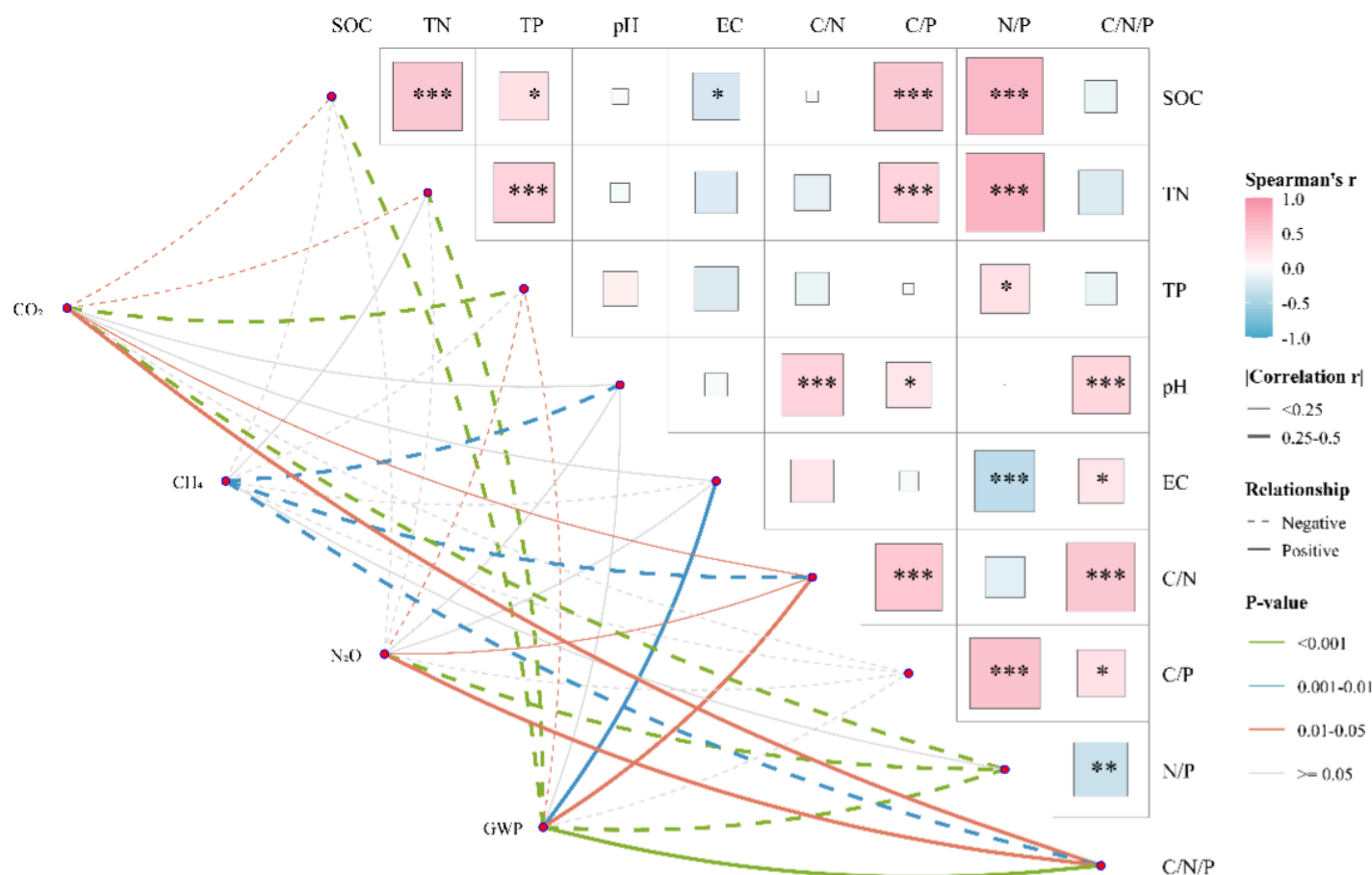


Figure 4

Relationships among GHG emissions, GWP, soil physicochemical properties, and nutrient stoichiometric characteristics in the maize planting system. Mantel tests and Spearman correlation analyses reveal relationships among soil GHG emissions (CO₂, CH₄, and N₂O), GWP, and soil physicochemical properties and nutrient stoichiometric ratios. CO₂: carbon dioxide; CH₄: methane; N₂O: nitrous oxide; GWP: global warming potential; EC: electrical conductivity; SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; C/N: carbon-to-nitrogen ratio; C/P: carbon-to-phosphorus ratio; N/P: nitrogen-to-phosphorus ratio; C/N/P: carbon-to-nitrogen-to-phosphorus ratio. The same applies below

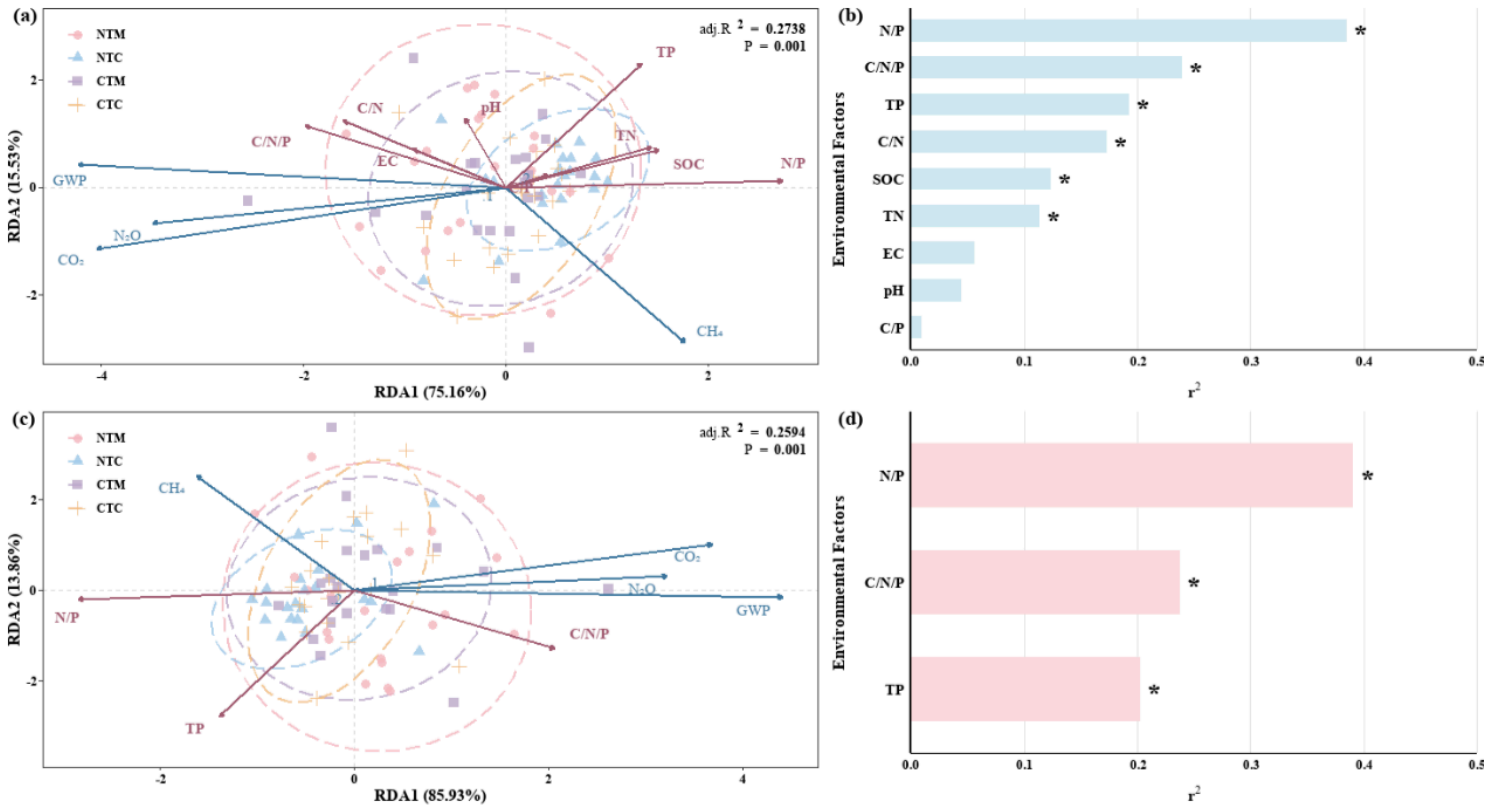


Figure 5

Redundancy analysis (RDA) showing the relationships between soil GHG emissions and soil environmental factors. (a) RDA ordination biplot of the full model including all measured variables. (b) Bar plot illustrating the explanatory power and statistical significance of each factor in the full model. (c) RDA ordination biplot of the parsimonious model obtained via forward selection. (d) Bar plot showing the contribution of the variance explained by the retained key factors in the parsimonious model. (Note: The full model includes pH, EC, SOC, TN, TP, C/N, C/P, N/P, and C/N/P. The parsimonious model was optimized using forward selection via the ordiR2step function, retaining four significant variables: N/P, TP, C/N, and TN. Blue and red arrows represent explanatory (environmental) and response (GHG) variables, respectively

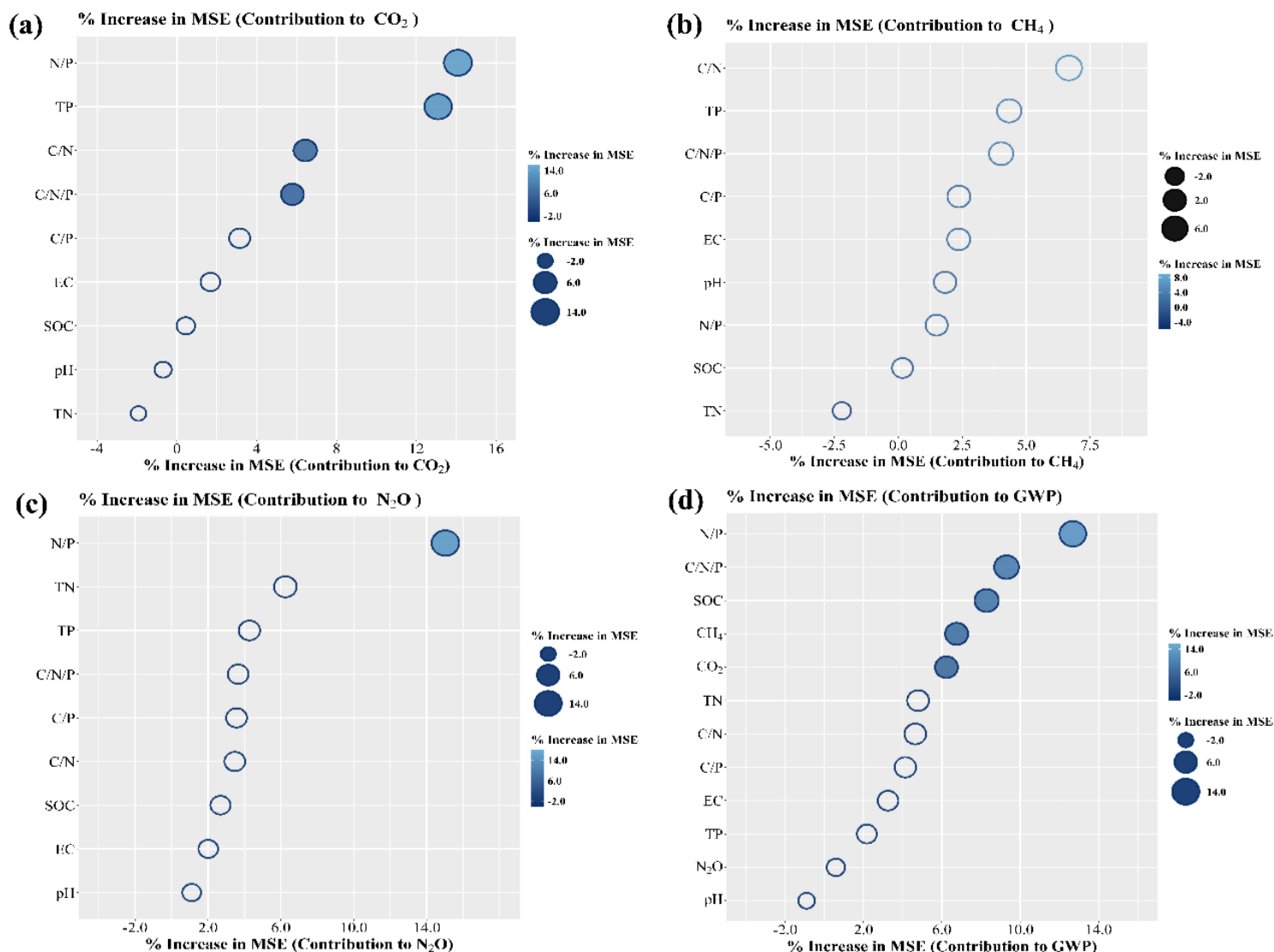


Figure 6

Random forest analysis to identify the main predictors of CO_2 (a), CH_4 (b), N_2O (c) and GWP (d) in the maize planting system

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