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Impact of soil moisture regimes on greenhouse gas emissions, soil microbial biomass, and enzymatic activity in long-term fertilized paddy soil

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Abstract

Two potent greenhouse gases that are mostly found in agricultural soils are methane and nitrous oxide. Therefore, we investigated the effect of different moisture regimes on microbial stoichiometry, enzymatic activity, and greenhouse gas emissions in long-term paddy soils. The treatments included a control (CK; no addition), chemical fertilizer (NPK), and NPK + cattle manure (NPKM) and two moisture regimes such as 60% water-filled pore spaces (WFPS) and flooding. The results revealed that 60% water-filled pore spaces (WFPS) emit higher amounts of N_2O than flooded soil, while in the case of CH_4 the flooded soil emits more CH_4 emission compared to 60% WFPS. At 60% WFPS higher N_2O flux values were recorded for control, NPK, and NPKM which are 2.3, 3.1, and 3.5 μg kg^{-1}, respectively. In flooded soil, the CH_4 flux emission was higher, and the NPKM treatment recorded the maximum CH_4 emissions (3.8 μg kg^{-1}) followed by NPK (3.2 μg kg^{-1}) and CK (1.7 μg kg^{-1}). The dissolved organic carbon (DOC) was increased by 15–27% under all flooded treatments as compared to 60% WPFS treatments. The microbial biomass carbon, nitrogen, and phosphorus (MBC, MBN, and MBP) significantly increased in the flooded treatments by 8–12%, 14–21%, and 4–22%, respectively when compared to 60% WFPS. The urease enzyme was influenced by moisture conditions, and significantly increased by 42–54% in flooded soil compared with 60% WFPS while having little effect on the β-glucosidase (BG) and acid phosphatase (AcP) enzymes. Moreover DOC, MBC, and pH showed a significant positive relationship with cumulative CH_4, while DOC showed a significant relationship with cumulative N_2O. In the random forest model, soil moisture, MBC, DOC, pH, and enzymatic activities were the most important factors for GHG emissions. The PLS-PM analysis showed that soil properties and enzymes possessed significantly directly impacted on CH_4 and N_2O emissions, while SMB had indirect positive effect on CH_4 and N_2O emissions.

Keywords Greenhouse gas emissions, Microbial biomass stoichiometry, Soil enzymes, Moisture content, Long-term fertilization

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Introduction

The emission of greenhouse gases (GHGs), which are released from the agriculture sector, is an important issue in research on total global warming GHG emissions. The total agricultural emissions are approximately 10–12% of the global anthropogenic greenhouse gas emissions [1–3]. China emits approximately 40% of atmospheric CH_4 and 60% of N_2O, accounting for approximately 17% of global emissions. Furthermore, 50% of methane and 25% of nitrous oxide emissions are produced by the agricultural sector [4, 5]. In the agriculture sector, the primary source that emits these greenhouse gases is paddy soil [6, 7]. The total paddy soil area in China is approximately 3×10^7 hectares, emitting 7.7–8.0 Tg methane and 138–154 Gg nitrous oxide per year [8, 9].

Soil moisture is a significant factor that affects N_2O and CH_4 emissions in soils. A typical soil moisture indicator is the water-filled pore space (WFPS), which provides extensive information on the water content and total porosity of the soil system [10]. Different irrigation methods, especially alternating wetting and drying methods, have a great impact on soil moisture, which in turn plays an important role in methane and nitrous oxide emissions [10, 11]. Methanogenesis, which occurs in anaerobic situations, and methanotrophy, which arises in aerobic environments, are the two main mechanisms that determine methane emissions [8, 12]. Flooded soils create anaerobic conditions for methanogenic bacteria, while an aerobic environment is produced during the drying process, resulting in methane oxidation [12]. During rice farming with the alternating wetting and drying irrigation method, soil moisture remains elevated, potentially creating a constrained anaerobic environment for methane formation, particularly in the deep soil profile [13, 14].

Compared to upland soil, flooded soils also differ in the production of nitrous oxide due to variations in oxygen (O_2) concentrations. The anaerobic environment of paddy soil limits the processes of mineralization, nitrification, and denitrification [15]. In long-term experiments, continuous flooding induces a serious anoxic environment in soils, resulting in complete denitrification and low N_2O fluxes. According to studies by Xu et al., higher emissions of nitrous oxide are released when WFPS increases from 45 to 90% [16]. The nitrification process dominates the production of nitrous oxide when the WFPS increases from 60 to 70%. Amnat et al. found that soils that produce N_2 limit nitrous oxide when WPFS exceeds 80% [17]. Wetland soil generates methane through methanogenesis, which occurs under anaerobic conditions during the decomposition of organic matter. Soil moisture actively maintains the moisture content at a precise level or rewets soils [18]. We need to evaluate how soil moisture affects CH_4 and N_2O emissions, soil microbial stoichiometry, and soil enzymes to figure out how different levels of moisture change the microbial biomass stoichiometry and enzyme activity in paddy soil and how these things relate to greenhouse gas (CH_4 and N_2O) soil properties and soil activities. The availability of soil nutrients, which are microorganisms' primary energy source, controls microbial biomass stoichiometry [19, 20]. The stoichiometry of soil microbial biomass indicates these microorganisms' respective nutritional requirements for growth [21]. Mooshammer et al. compare this stoichiometry to readily available soil resources to determine if the microorganisms' nutrient needs balance with the availability of nutrients in their surroundings. If there is a stoichiometric imbalance in the supply and demand of resources, a specific nutrient may limit microbial activity [22, 23]. Nutrient supplementation can reduce imbalances in C: N, C: P, or N: P ratios, thereby reducing microbial nutritional constraints [24]. The availability of soil nutrients, according to ecological stoichiometric theory, has a direct impact on microbial activity and growth. In other words, nutrients limit microbial growth [25]. Long-term fertilization application increases nutrient availability, especially nitrogen and phosphorous, which boosts soil microbial biomass and may reduce nutrient stoichiometry [25, 26]. By synthesizing extracellular enzymes, soil bacteria can get limited nutrients from the decomposition of soil organic materials [27, 28]. As a result, variations in enzyme activity can reflect nutritional restriction in soil bacteria to some degree [28]. Here, we evaluated the impact of moisture regimes on methane and nitrous oxide fluxes, microbial biomass stoichiometry, and enzymatic activities. Specifically, we tested the following hypotheses: (1) find out how the soil moisture content affects the stoichiometry of microbial biomass succession; (2) uncover the rate of GHG (CH_4 and N_2O) emissions under different moisture conditions; and (3) explore the dynamics of enzymatic activity in paddy soils.

Methods and materials

Soil samples, location, and properties

We took soil samples from the farming experimental station at Qiyang, southern China, at the National Observation and Research Station (26°45′42" N, 111°52′32" E), at a height of approximately 160 m above sea level. The annual average temperature of the air is between − 8.4 and 40 °C, and approximately 1259 mm of precipitation. This experiment started in 1982 and the cropping system was early rice, late rice, and winter fallow. We select three different fertilization treatments: control (CK), chemical fertilizer (NPK),

and NPK fertilizer plus cattle manure (NPKM) (NPKM = NPK + 22.5 t/ha fresh cattle manure). All the treatments had three replicates followed by a random block group arrangement. The soil, which was initially formed from quaternary red clay, is a typical ultisol with low fertility. Its pH is 5.97, and its SOC content is 12.2 g kg^{-1}. In the original soil, TN, TP, TK, AN, AP, and AK in the original soil were 1.5 g kg^{-1}, 0.48 g/kg, 14.2 g kg^{-1}, 158 mg kg^{-1}, 9.6 mg kg^{-1}, and 65.9 mg kg^{-1}, respectively. We collected soil samples from three distinct locations within each plot, ranging in depth from 0 to 20 cm. Afterward, the soil samples were air-dried, pulverized, and sieved through a 2-inch mesh sieve.

Incubation and treatment details

The sieved soil was pre-incubated at 30–40% water-holding capacity and maintained at 25 °C in an incubator for one week. The purpose of pre-incubation was to stabilize the soil microbial activity. We took 200 g of sieved soil from every field and placed it in a 1-L glass jar. The collected soils had been treated with two levels of moisture (60% and flooding) [30] with long-term fertilization treatments (CK, NPK, NPKM) and were mixed well. To calculate the daily flux rates for nitric oxide and methane emissions, One-liter glass bottles containing 200 g of soil were filled and placed to incubate for 60 days at 25 °C. Before gas sampling, the glass jars were covered with a polyethylene to reduce moisture loss and permit gas exchange. The jars were weighed daily to keep the moisture constant, and distilled water was added. Separate sets of soil samples were generated to collect the gas samples and analyze soil characteristics. The physical and chemical parameters of the soil samples were examined after completing the incubation study.

N_2O and CH_4 emission sampling method

Gas samples were regularly taken from the jars for the first eight days and then at two-day intervals from the 9th to the 17th. From the 18th to the 32nd day, gas samples were taken from each treatment after four-day intervals and after the 32nd day once a week until the end of the incubation study. At 0, 12, and 24 h following the beginning of the test, gas samples were extracted from the jars containing each treatment. The glass jars were left open for thirty minutes to let outside air into them before beginning the gas sample. The jars were then closed with corks of rubber, fitted with three-way valves. Gas samples were taken at two different times (0 and 1 h) using a 50 ml gas-tight syringe (BD Luer-LokTM Tip, China). An Agilent greenhouse gas chromatograph (7890 GC, Agilent Technologies Australia) was used to analyze the nitrous oxide and methane emission samples immediately after they were collected. The equation that was used for calculating the fluxes was provided by [29].

$$F = \rho \times \Delta C \times V \times \left(\frac{273}{273 + T} \times W^{-1} \right),$$

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

where F denotes the nitrous oxide (μg/kg soil/h) and methane (μg/kg soil/h) emissions; ρ represents the density of a gas at room temperature. The variables W, T, V, and ΔC represent the weight (kg) and gas space volume (m^3) of the soil used in the experiment, as well as the change in gas concentration between 0 and 2 h of incubation. Fi and Fi + 1 represent the methane and nitrous oxide emission rates at times ti and ti + 1, respectively, while E represents the total methane and nitrous oxide emissions.

Laboratory analysis of soil

After 60 days of incubation, 5 g of fresh soil samples was extracted with 0.05 M K_2SO_4 agitated for an hour, filtered using Whatman #42 filters, and tested for exchangeable ammonium and nitrate using a flow injection analyzer (Lachat Instruments, Loveland, CO, USA). The incubated soil's pH was measured using a pH meter at a ratio of 1:2.5 for soil to deionized water. The potassium dichromate method was utilized for the determination of organic carbon [30]. Available phosphorus was measured following Murphy`s procedures [31].

For the determination of microbial biomass phosphorus (MBP), nitrogen (MBN), and carbon (MBC), we used the fumigation method [32, 33]. For the precise measurement of MBC, MBN, and MBP, incubated soil samples were subjected to chloroform for 24 h. To determine the carbon and nitrogen microbial biomass, fumigated and non-fumigated soil samples were extracted using 0.05 M K_2SO_4, shaken for an hour, filtered, and then sent to TOC-VCPH for determination. The values of MBC and MBN were determined by dividing the extraction efficiency (0.5) by the variance among the levels in under fumigation and non-fumigated soils. Soil samples from fumigated and non-fumigated were mixed with 1 mL KH_2PO_4 (250 Gp m/L) for an hour before extraction to extract microbial biomass phosphorus. Using an extraction efficiency of 0.40, the MBP was computed. The soil microbial quotient (SMQ) was determined by applying the following formula: SMQ = SMBC/SOC. A microplate methodology was used to measure the activity of these extracellular enzymes. For urease enzymes, we used the method described by Yang et al. [34]. Acid phosphatase and β-glycosidase activities were measured

by the standard protocol of Tabatabai et al. [35]. The enzyme activity was measured in nmol/g soil/h.

Statistical investigation

We used SPSS software version 21 (IBM SPSS Statistics; Chicago, USA) for statistical analysis and Sigma Plot to generate data visualizations. The duplicated data were analyzed using a two-way ANOVA, with LSD testing used to find significant differences between the treatments and their interactions with N_2O, CH_4, and soil microbial biomass activity. We investigate relationships between soil properties using regression analysis. We performed a random forest analysis using the "random forest" package in R to evaluate the pivotal and credible predictors of soil greenhouse gases among different soil factors.

Results

Soil physiochemical properties

The chemical properties of soil that was treated with continuous fertilizer are listed in Table 1. Soil pH was significantly increased under flooding conditions compared with 60% WFPS after incubation for 60 days. The maximum pH of 5.89 was observed under flooding conditions in the NPKM treatment, while the lower pH of 5.3 was recorded at 60% WFPS in the control and chemical fertilizer treatments. The moisture had no significant effect on the SOC concentration, while significantly increased under long-term fertilization (without moisture treatment) and the highest SOC concentration was observed under NPKM followed by NPK and CK (Table 1). The DOC concentrations were significantly high with moisture and long-term fertilization. A high DOC content of 49.1 $mgkg^{-1}$ was recorded in the NPKM flooded treatment, while the lower (19.7 mg kg^{-1}) DOC was noted in the CK (60% WFPS) treatment. Different moisture regimes had non-significant effects on TP and AP, but under long-term fertilization, their effects were significant. Furthermore, the interactive effect between long-term fertilization and moisture was non-significant in all measured parameters.

Methane (CH_4) and nitrous oxide (N_2O) emissions

Soil CH_4 emissions were significantly affected by soil moisture content and long-term fertilization. The combined NPK and manure (NPKM) treatments significantly increased CH_4 emissions in flooded soil conditions compared with the CK treatment, and a decreasing trend was observed throughout the incubation period at 60% WFPS. Maximum CH_4 fluxes of 3.8 μg kg^{-1} h^{-1}, 3.2 μg kg^{-1} h^{-1}, and 1.7 μg kg^{-1} h^{-1} were noticed on day 24 in NPKM, NPK, and CK flooded treatments, respectively (Fig. 1). Similarly, the 60% WFPS treatment, overall, showed less CH_4 emissions such as on day 13 the NPKM emits 1.34 μg kg^{-1} h^{-1}, whereas on day 11 small peak was also observed in CK (1.1 μg kg^{-1} h^{-1}) and NPK (1.19 μg kg^{-1} h^{-1}) (Fig. 1). However, the cumulative CH_4 emissions were significantly increased in all treatments of flooded soil compared with 60% WPFS soil. The maximum cumulative emissions were recorded in the fertilized treatments in flooded soil compared with the control (Fig. 2).

The peaks of N_2O emissions in all treated soils were higher at the start, and later followed a decreasing trend till the end of the incubation. Water regimes and long-term fertilizations both had a significant impact on N_2O emissions. Figure 1 shows that 60% of WFPS treatments emit higher N_2O emissions than flooded soil. On day 4 all the treatments (CK, NPK, and NPKM) in 60% moisture showed the highest peaks of 1.4, 6.7,

Table 1 Soil chemical properties influence at 60% WFPS and flooding condition after various fertilization regimes

Treatments	WFPS	pH	SOC $g{\cdot}kg^{-1}$	DOC mgkg − 1	TP gkg^{-1}	AP $mgPkg^{-1}$	NO_3 $mgNkg^{-1}$	NH_4 $mgNkg^{-1}$
CK	60%	5.38 ± 0.09c	16.1 ± 2.6c	19.7 ± 2.7e	1.33 ± 0.21b	8.44 ± 1.6c	1.41 ± 0.1c	6.7 ± 0.57d
NPK	60%	5.3 ± 0.11c	21.3 ± 2.3b	33.5 ± 2.6 cd	1.95 ± 0.18a	31.1 ± 3.2b	1.92 ± 0.21b	9.2 ± 2.3bcd
NPKM	60%	5.43 ± 0.19bc	29.8 ± 1.9a	41.5 ± 4.2b	2.27 ± 0.26a	46.8 ± 5.2a	2.2 ± 0.21ab	11.4 ± 3.2ab
CK	Flooding	5.78 ± 0.14a	18.2 ± 3.1bc	27.1 ± 3.7d	1.34 ± 0.22b	9.3 ± 1.7c	1.37 ± 0.14c	7.8 ± 0.59 cd
NPK	Flooding	5.69 ± 0.21ab	22.1 ± 2.5b	39.7 ± 4.1bc	1.99 ± 0.21a	31.8 ± 3.6b	1.79 ± 0.21ab	11.3 ± 1.9abc
NPKM	Flooding	5.89 ± 0.11a	31.2 ± 3.1a	49.1 ± 6.3a	2.31 ± 0.24a	48.2 ± 7.3	1.9 ± 0.19a	14.1 ± 1.8a
Two-way ANOVA								
Fertilization		*	**	**	**	**	**	**
Moisture		**	ns	**	ns	ns	ns	ns
F*M		ns	ns	ns	ns	ns	ns	ns

CK (Control), NPK (Chemical Fertilizer), NPKM (Chemical Fertilizer and Chicken manure). Mean ± Standard deviation ($n = 3$)

SOC soil organic carbon, *DOC* dissolve organic carbon, *TP* total phosphorus, *AP* available phosphorus, NO_3 nitrate, NH_4 ammonium

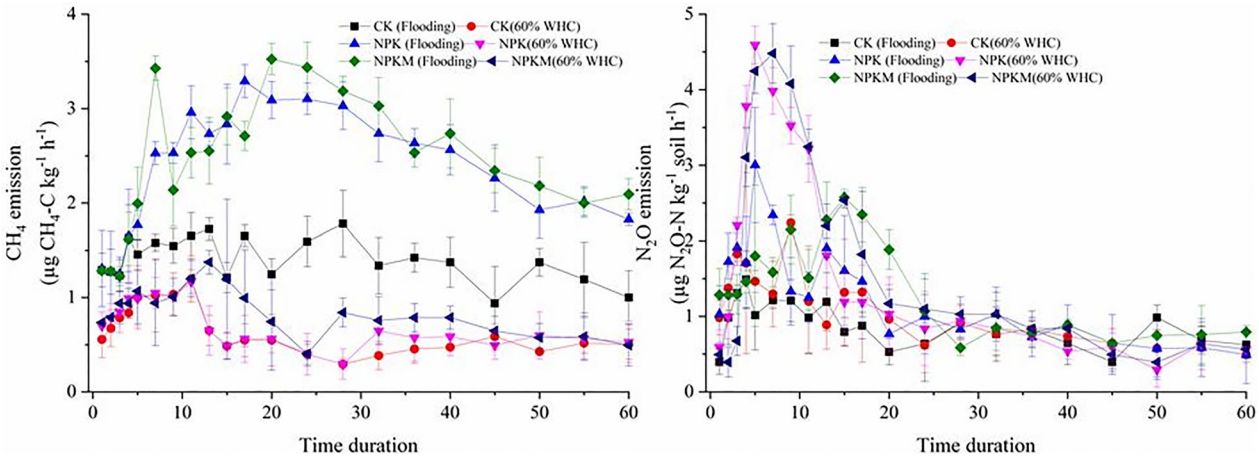

Fig. 1 CH_4 and N_2O emission fluxes in unfertilized and fertilized soils under 60% WFPS and flooded condition. Different letters show significantly different means at $p < 0.05$. Standard error is shown by bars; $n = 3$

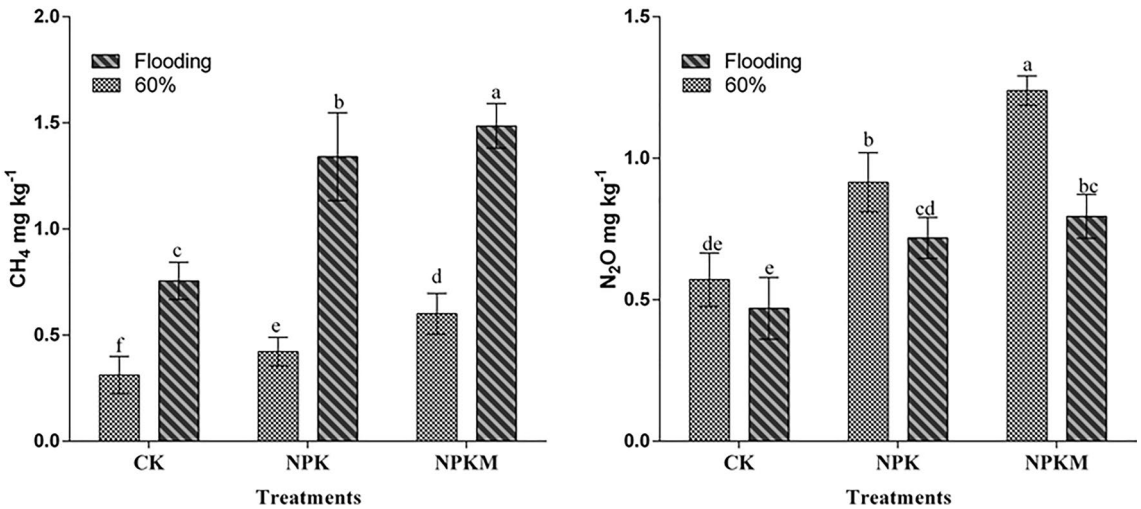

Fig. 2 Cumulative CH_4 and N_2O emission in long-term fertilized and unfertilized soil under 60% WFPS and flooded condition. Significantly different means at $p < 0.05$ are represented by different letters

and 7.1 μg kg-1 h-1, respectively (Fig. 1). Consequently, the N_2O showed peaks till 10th day, while later showed no significant peak through the study period of 60 days. Similarly, in case of flooding treatment small peaks of 2.3, 3.1, and 3.5 μg kg^{-1} h^{-1} in the CK, NPK, and NPKM treatments, respectively, were observed. Cumulatively, the highest N_2O emissions were recorded in NPKM (1.2 mg kg^{-1} h^{-1}) at 60% WFPS, while the lowest N_2O (0.4 mg kg^{-1}) in flooded soil in CK treatment.

Soil microbial biomass stoichiometry and activity

All the microbial biomasses of carbon, nitrogen, and phosphorus (MBC, MBN, and MBP) significantly increased with different moisture regimes to long-term fertilized paddy soil. Compared with the 60% WFPS conditions, the flooded conditions significantly increased (Table 2). The MBC concentration in flooded treatments was 8–12% higher compared to 60% WFPS conditions. Under 60% WFPS conditions, the MBC values ranged from 117 to 160 mg C kg^{-1} (Table 2). Under long-term fertilization, the NPKM treatment showed higher MBC concentration of 182 and 160 mg C kg^{-1} for flooding and 60% WFPS soil, respectively (Table 2). Similarly, the MBN concentration in the present study was increased under both flooding and 60% WFPS treatment, particularly increased in flooding treatment which showed 14–21% higher MBN concentration that 60% WFPS. In case of MBP the flooding treatment (15–44 mg P kg^{-1}) revealed

Table 2 Effect of soil moisture on soil microbial biomass stoichiometry after long-term fertilization

Treatments	WFPS	MBC $mgCkg^{-1}$	MBN $mgNkg^{-1}$	MBP $mgPkg^{-1}$	MBC:MBN $mgkg^{-1}$	MBC:MBP $mgkg^{-1}$	MBN:MBP $mgkg^{-1}$	MQ %
CK	60%	117.7 ± 6.5d	24.2 ± 4.9e	15.2 ± 3.9e	5.1 ± 0.89a	8.1 ± 1.2ab	1.64 ± 0.22a	0.73 ± 0.09ab
NPK	60%	139.8 ± 21.2bc	39.2 ± 6.5cd	24.5 ± 4.1cd	3.5 ± 0.42abc	5.7 ± 0.42bc	1.6 ± 0.11a	0.66 ± 0.16abc
NPKM	60%	160.3 ± 10.2b	53.3 ± 8.1b	34.3 ± 5.4b	3.02 ± 0.43bc	4.7 ± 0.53c	1.58 ± 0.31a	0.53 ± 0.04c
CK	Flooding	132.4 ± 8.9cd	30.9 ± 5.5de	15.8 ± 3.6de	4.5 ± 0.98ab	8.7 ± 1.2a	1.94 ± 0.24a	0.79 ± 0.07a
NPK	Flooding	153.5 ± 7.7bc	46.4 ± 7.4bc	31.1 ± 6.0bc	3.3 ± 0.32bc	5.1 ± 0.89c	1.61 ± 0.09a	0.70 ± 0.09abc
NPKM	Flooding	182.9 ± 12.5a	67.7 ± 4.2a	44.4 ± 6.6a	2.7 ± 0.28c	4.1 ± 0.61c	1.54 ± 0.14a	0.58 ± 0.04bc
Two-way ANOVA								
Fertilization		**	**	**	**	**	ns	*
Moisture		*	*	*	ns	ns	ns	ns
F*M		ns	Ns	ns	ns	ns	ns	ns

CK (Control), NPK (Chemical Fertilizer), NPKM (Chemical Fertilizer and Chicken manure). Mean ± Standard deviation ($n = 3$)

MBC microbial biomass carbon, *MBN* microbial biomass nitrogen, *MBP* microbial biomass phosphorus, *MQ* microbial quotient

Note: **: p ≤ .01; *: p ≤ .05; ns: not significant

higher MBP compared to 60% WFPS (15–34 mg P $kg^{-}1$) (Table 2), which is 4–22% higher than 60% WFPS soil. Interestingly, the microbial quotient increased in flooded soil compared with 60% WFPS soil, while it decreased in fertilized soil compared with the control (Table 2); also, there was a non-significant change found between moisture regimes and microbial biomass stoichiometry (Table 2).

Soil extracellular enzymatic activities varied between moisture regimes and long-term fertilization (Fig. 3). However, under flooded conditions, the urease activity significantly increased by 42–54% compared to 60% WFPS conditions. Under long-term fertilization, the NPKM treatment significantly increased β-glycosidase and acid phosphatase (BG and AP) enzyme activities, whereas the moisture content had little effect on BG and AP, which was 1.2–6.1% and 2–6.6%, respectively (Fig. 3). Furthermore, the highest values were observed for urease, BG, and AP in NKPM treatment.

Relationship between soil properties and enzymatic activities with cumulative CH_4 and N_2O emissions

In flooded and 60% WFPS soil, Figure S1 shows the Pearson correlation of microbial stoichiometry and soil properties with greenhouse gases. A linear relationship was identified between CH_4 and N_2O and other soil-related parameters. The DOC also exhibited a significant positive relationship with cumulative N_2O, while MBC and pH revealed a non-significant relationship with cumulative N_2O emissions (Fig. S2). Accordingly, cumulative CH_4 emissions were significantly correlated with DOC by $R^2 = 0.55$ ($p < 0.05$), with SMBC by $R^2 = 0.62$ ($p < 0.05$), and with pH by $R^2 = 0.67$ ($p < 0.05$) (Fig. S2). Soil extracellular enzymatic activities of urease, β-glycosidase, and acid phosphatase had a significant effect on N_2O, while there was a non-significant effect on CH_4 (Fig. S3).

Regulation of CH_4 and N_2O emissions

We conducted random forest analysis to explore the relative importance of soil properties and microbial activities on greenhouse gas emissions (CH_4 and N_2O), and we conducted random forest analysis. In terms of soil properties, soil moisture, MBC, DOC ($p < 0.001$), and pH were the most significant factors for CH_4 and N_2O emissions (Fig. 4a). The DOC, NH_4^+, and NO_3^- concentrations ($p < 0.01$) were also important variables for GHG emissions. Furthermore, the activities of urease enzymes ($p < 0.001$) and the microbial quotient (MQ) ($p < 0.001$) were the most important factors for CH_4 and N_2O emissions, respectively (Fig. 4b). β-glycosidase and MBC:MBN ($p < 0.01$) were also vital signs for CH_4 diffusive flux, whereas β-glycosidase and urease ($p < 0.01$) were identified as important variables for N_2O diffusion.

Direct and indirect effects of soil properties, microbial biomass, and activities on CH_4 and N_2O emissions

To explore the role of different explanatory indicators and complex interrelationships in the emissions of CH_4 and N_2O, we employed a partial least squares path model (PLS-PM) (Fig. 5). The results showed that the soil properties (0.43) and enzymes (0.46) had a prominently advantageous direct impact on GHGs (CH_4 and N_2O). Furthermore, the indirect influence of soil microbial biomass (0.43) proved to be more significant and beneficial than of soil properties (0.35) on GHG emissions, which was positively associated with soil enzymes (β-glycosidase, AcP, and urease). Moreover, soil properties (0.84) had an important direct effect on the

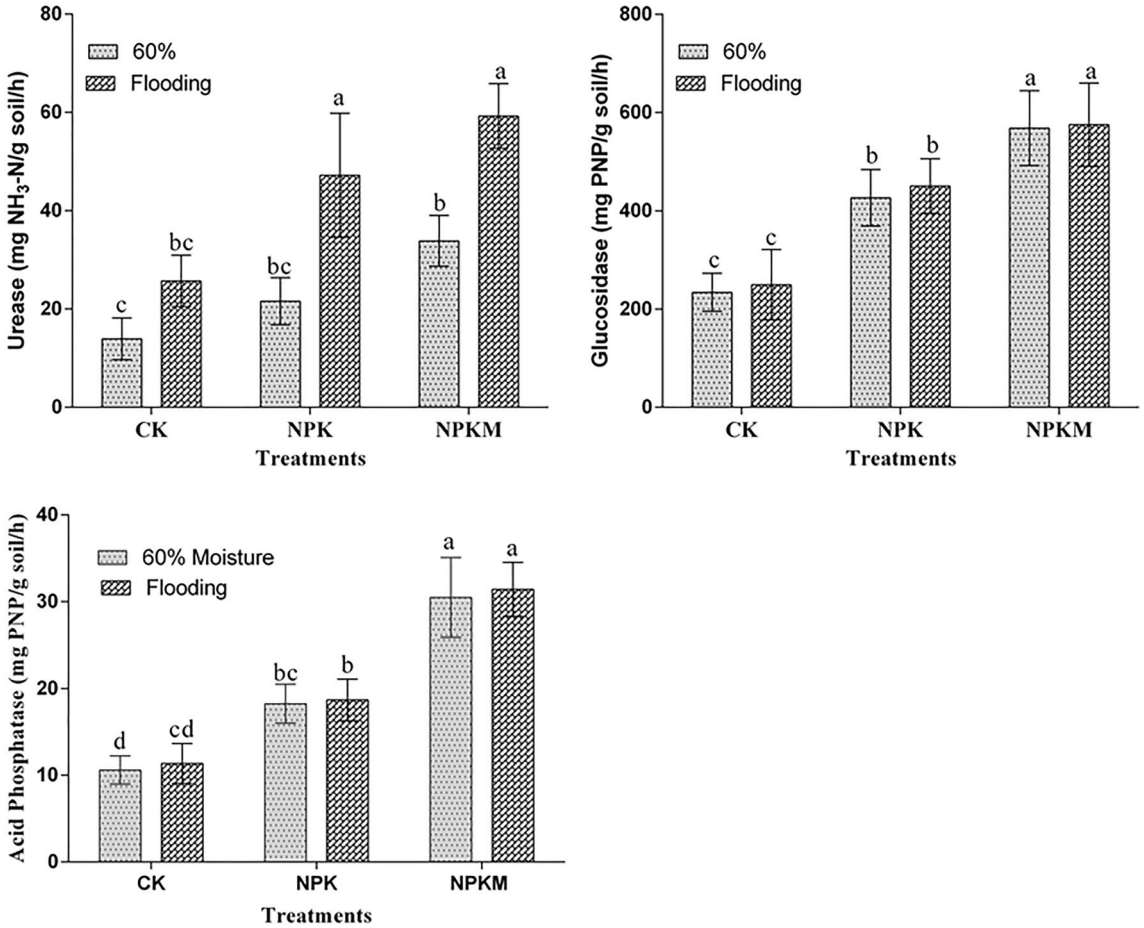

Fig. 3 Response of soil enzymes to long-term fertilization under two moisture levels. Significant differences between the treatments are shown by different lowercase letters at $P < 0.05$. *β-glycosidase* Glucosidase, *AcP* Acid Phosphatase

soil microbial biomass. However, the direct effect of soil microbial biomass on GHG emissions was not significant. The loading scores suggested that NO_3^-, MBC:MBN, and urease were the most potent indicators of soil properties, soil microbial biomass, and soil enzymes, respectively, compared with other prospects for that potential variable.

Discussion

Paddy soil contributes to methane emissions, accounting for 31–112 Tg/y yearly, or 9–19% globally [36]. Soil moisture is one of the main factors that drives methane production and has a crucial effect on CH_4 formation. This study showed that methane fluxes were lower in wet soil and higher in methane emissions than 60% WFPS. Our findings are consistent with previous research, which found that a high soil moisture level increased methane fluxes [37]. Higher levels of water-filled pore space in soil led in higher emissions of methane [38, 39]. In a recent study, methane fluxes were high compared to the 60% WFPS moisture in this study. The methanogenic activities in soil rise with soil moisture content, but methanotroph activities drop with soil oxidized zone reduction [40]. Increased soil moisture caused anaerobic conditions in the present study which promoted the activities of methanogenic rather than methanotrophic bacteria, resulting in the detected methane fluxes [41]. Increased soil moisture content assists in the breakdown of native SOM, which acts as a stimulant for methanogens to produce CH_4 [40, 41].

The presence of both aerobic and anaerobic microsites in the soil is directly correlated with its water content; about 60–70% WFPS provides suitable conditions to assist nitrification and denitrification simultaneously and thus produce more N_2O emissions [42]. According to current findings, the increase in N_2O emissions in 60% WFPS soil was likely due to higher soil nitrate concentration and the reduction in NH_4^+-N during nitrification processes in all treatments. Microbial nitrification and denitrification processes consume soil

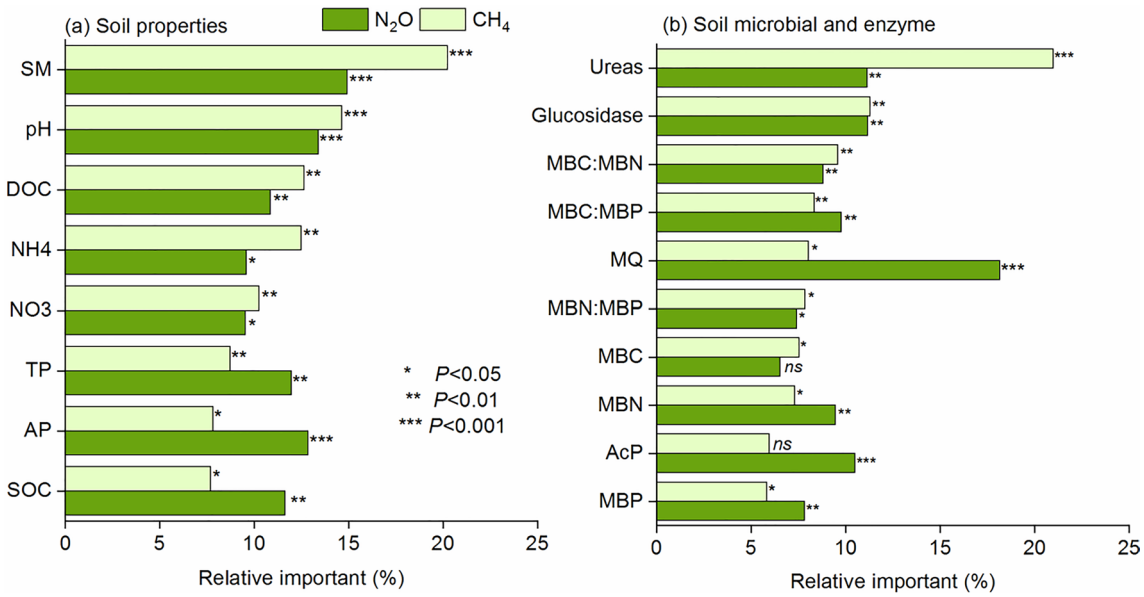

Fig. 4 The relative important (%) of predictor variables for the random forest model of cumulative CH_4 and N_2O emission. *SM* soil organic matter, *DOC* dissolve organic carbon, *NO*$_3$ nitrate, *NH*$_4$ ammonium, *TP* total phosphorus, *AP* available phosphorus, *SOC* soil organic carbon, *Glucosidase* β-glycosidase, *MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, *MBP* microbial biomass phosphorus, *MQ* microbial quotient, *AcP* acid phosphatase

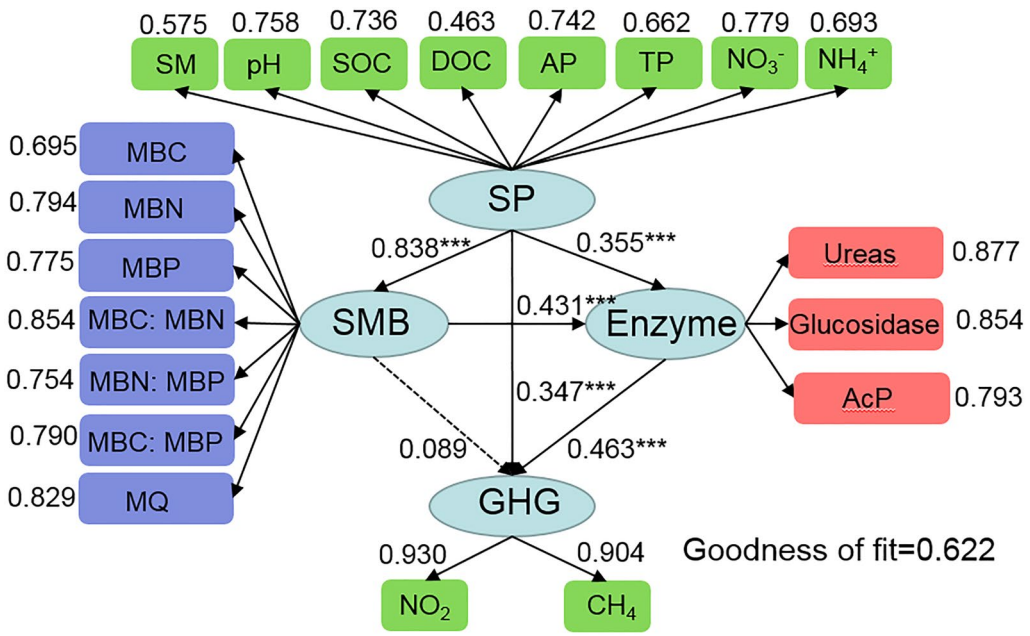

Fig. 5 Partial Least Squares Path Model (PLS-PM) and the effect of soil property legacy effect on GHG emissions and the related microbial stoichiometry during the study period. Each box represents observed variables or latent variables. Larger path coefficients are reflected in the width of the arrow with black indicating a positive effect. Path coefficients that were not significantly different from 0 are shown in black dashed lines. Path coefficients are calculated after 1000 bootstraps. The model is assessed using the Goodness of Fit statistic. *SM* soil organic matter, *DOC* dissolve organic carbon, *NO*$_3$ nitrate, *NH*$_4$ ammonium, *TP* total phosphorus, *AP* available phosphorus, *SOC* soil organic carbon, *Glucosidase* β-glycosidase, *MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, *MBP* microbial biomass phosphorus, *MQ* microbial quotient, *AcP* acid phosphatase

ammonium and nitrate nitrogen as substrates, producing nitrous oxide as an intermediate product, while at 60% WFPS soil moisture content, the nitrous oxide emissions decrease [43]. Higher water-filled pore space improves anaerobic soil conditions, encouraging denitrification, and converting nitrous oxide into nitrogen, resulting in lower or no nitrous oxide emissions [44–46]. In flooded soil, denitrification is a crucial process that substitutes nitrogen oxides with oxygen as an electron acceptor. Current findings revealed that at 60% WFPS, more nitrous oxide was produced than in flooded soil (Figs. 1, 2). Flooding consistently creates an anaerobic environment in the soil and alters the chemical and biological processes that limit organic carbon and nitrogen mineralization, subsequently lowering substrates for N_2O emissions [47]. Our results are consistent with the findings of Shang et al. who reported low N_2O emissions from flooded soils [48]. The previous study's findings observed insignificant N_2O emissions from flooded soils, which aligns with our results. Flooded soils are usually referred to as anaerobic because the water-filled soil pores limit the oxygen available [48]. The explanation for this is that the majority of denitrifying bacteria are facultative anaerobes, meaning they prefer to accept oxygen as an electron acceptor but will also absorb nitrogen oxides as an electron acceptor if oxygen becomes scarce [48, 49]. N_2O emissions increased during the incubation study's early phases and reduced as it carried on.

According to Fig. 2 of the current investigation, N_2O emissions were slightly lower in the CK treatment than in the NPK and NPKM treatments. According to Shaaban et al. (2015), N_2O emissions were much lower at 55% WFPS than at 90% WFPS [45]. The amount of SOC breakdown produced by variable soil moisture levels might explain the differences in cumulative N_2O emissions between moisture level treatments [49]. Furthermore, the 60% WFPS treatment provided aerobic soil conditions, but the flooding conditions created anaerobic soil conditions. Increasing the moisture level of soil from 60 to 100% resulted in a significant reduction in nitrous oxide (Fig. 2), which is consistent with previously reported findings [50].

Soil moisture plays an important role in regulating N_2O emissions [51]. Results showed that the CK treatment significantly reduced N_2O emissions compared to the NPK and NPKM treatments (Fig. 2). The main reason for the differences in total N_2O emissions between treatments was the different amounts of SOC breakdown caused by different soil moisture levels [50, 51]. In addition, there were aerobic conditions under 60% WFPS, while flooding under WFPS resulted in anaerobic conditions. It is well established that repeated aerobic and anaerobic conditions result in nitrification and denitrification, respectively [51, 52]. A previous study revealed that lower N_2O emissions were produced under nitrification than denitrification [46]. Our findings demonstrated that increasing the soil moisture content from 60 to 100% WFPS resulted in a considerable reduction in N_2O emissions (Fig. 2), which is consistent with prior research findings.

Additionally, soil moisture contributes to the breakdown and solubilization of organic carbon, which releases readily accessible carbon and acts as a precursor for the development and metabolism of soil microorganisms [53]. The dissolved organic carbon and microbial biomass carbon stocks were greater under flooding conditions than under 60% WFPS (Tables 1, 2), showing that soil moisture promoted the solubilization and decomposition of indigenous organic matter. The favorable relationship among CH_4 emissions, DOC, and MBC was further highlighted by correlation analysis. The results revealed that moisture content elevated soil pH from 5.3 to 5.7, 5.6, and 5.8 in the CK, NPK, and NPKM treatments, respectively. On the other hand, the soil pH of the 60% WFPS treatments declined throughout the incubation period. The current findings align with a recent study, which showed that the significant pH decline in soil originates from the transformation process, which produces two moles of protons for every molecule of NH_4^+ oxidized to NO_3 [54].

One important measure for assessing the quality of soil is the biomass of soil microbes. SMB is less resistant to soil management practices and environmental variables than soil organic matter [55]. Although SMB is a small portion of OM, it plays a critical role in processes such as soil nutrient cycling and the transformation of soil organic matter and insoluble materials [44–46]. Similar to the findings of the present study, the enriched stocks of microbial biomass under long-term fertilization have been found to exhibit increased metabolic activity of microorganisms [56]. Accordance to previous studies, long-term inorganic fertilization combined with manure (NPKM) increased microbial biomass carbon and nitrogen as compared to the inorganic fertilizer and control treatments (Table 2). According to previous research, anthropogenic C input has effectively increased microbial biomass carbon and nitrogen due to the strong activation of microbes under high soil carbon concentrations [27]. Lower microbial biomass ratio between microbial biomass carbon and phosphorus may drive soil microorganisms to release nutrients and increase the availability of nitrogen and phosphorus pools [57, 58]. The findings of the present study also found a strong link between microbial biomass stoichiometry and nutrient inputs

in the soil as described by [59]. In this study, compared with the NPK and NPKM treatments, the control (CK) treatment intensely reduced MBP (Table 2). Dai et al. (2019) discovered in an earlier investigation that N: P stoichiometry is the primary regulator of microbial biomass phosphorus. They also reported that adding phosphorus over time improved microbial phosphorus immobilization by lowering the relative abundance of phosphorus-depleted microbial communities [60].

The current study found that combining organic and inorganic fertilizers increased the soil SOC content, which influenced soil available nutrients and microbial biomass stoichiometry and its ratios (Table 2). These findings are in line with earlier research on the issue [59–61]. Additionally, in the present study, the control treatment substantially reduced MBP compared to the NPK and NPKM treatments (Tables 1 and 2).

Compared with the NPK and CK treatments, the application of inorganic fertilizers combined with manure (NPKM) caused the greatest increase in enzyme activities (Fig. 4) [59]. According to a prior study, pig dung combined with inorganic fertilizers substantially boosted extracellular enzymatic function in rice when compared with sole inorganic fertilizers and sole manure applications [61]. Increased soil acidity could contribute to reduced enzyme activity when applying inorganic fertilizer. Inorganic nitrogen input lowered soil pH, which resulted in lesser soil microbial activity and influenced the quantity of the phosphatase-solubilizing microbial community [60–62].

Conclusion

The findings of this study reveal that variation in moisture has a significant impact on both GHG emissions. Flooded soil resulted in lower N_2O emissions than 60% WFPS and high CH_4 emissions are found in flooded soil as compared to 60% WFPS, while long-term organic and inorganic treatments showed higher emissions in both water regimes. Increasing soil moisture content raised pH levels, which in effect raised nosZ gene transcripts and reduced soil emissions of nitrogen oxides. Cumulative CH_4 emissions had a substantially enhancing effect on DOC, MBC, and pH, while cumulative N_2O emissions had a favorable effect on soil enzymes. Our findings suggest that moisture is an important factor that affects GHG fluxes, soil nutrient availability, and activities. Therefore, we needed further research to understand the mechanism of methane, which involves the activities of methanogenesis and methanotrophs in different soil moisture levels.

Supplementary Information

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Supplementary Material 1.

Author contributions

AS contributed to conceptualization, data curation, formal analysis, methodology, writing—original draft, and writing—reviews and editing. HZ was involved in conceptualization, resources, writing—review and editing, and supervision. MNK performed data curation and methodology. KAT contributed to resources and methodology. TH contributed to formal analysis and resources. MFS and SF were involved in writing—review and editing. JH performed investigation and writing—original draft preparation. NAD was involved in writing—reviews and editing. SK performed writing—review and editing.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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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