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Comparison of Nitrous Oxide Consumption of Paddy Soils Developed from Three Parent Materials in Subtropical China

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Abstract: Paddy soils developed from various parent materials are widely distributed in the subtropical region in China and have a non-negligible but unclear potential to consume nitrous oxide (N₂O) due to long-term flooding. This study selected three of the most common paddy soils in subtropical China, developing from quaternary red soil (R), lake sediment sand (S), and alluvial soil (C), to study their total N₂O consumption and total nitrogen (N₂) production using N₂-free microcosm experiments. These paddy soils were treated with N₂O addition (N₂O treatment) or helium (He) addition (CK treatment) and incubated under flooding and anoxic conditions. The results showed that three alluvial soils (C1, C2, and C3) consumed over 99.93% of the N₂O accumulated in the soil profile, significantly higher than R and S soils ($p < 0.05$). And the N₂ production in three C soils was also significantly higher than other soils, accounting for 81.61% of the total N₂O consumption. The main soil factors affecting N₂O consumption in C, S, and R soils were soil clay content ($p < 0.05$), soil sand content ($R^2 = 0.95$, $p < 0.001$), and soil available potassium (AK) ($p < 0.01$), respectively. These results indicate flooding paddy soils, no matter the parent materials developed, could consume extremely large amount of N₂O produced in soil profiles.

Keywords: paddy soils; N₂O consumption; N₂ production; soil parent materials; flooding and anoxic



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1. Introduction

Paddy soil is an important and typical agricultural soil, with over 135 million hm² planting area worldwide, of which China accounts for 28% [1]. The special water management of rice during the growth period causes the soil to emit a large amount of nitrous oxide (N₂O) into the atmosphere [2], which is one of the three major greenhouse gases in the world [3]. Although soil is a net source of N₂O in most cases, researchers have found that soil can uptake N₂O from the atmosphere, resulting in negative N₂O emissions [4–6]. Chapuis-Lardy et al. [7] demonstrated that a negative emission of N₂O happens when the amount of N₂O reduction exceeds the production amount in soil. Majumdar et al. [8] reported that N₂O uptake in paddy soils ranged from 0.13 to 191 μg·m^{−2}·h^{−1}. It has also been shown that flooded or anaerobic environmental conditions are conducive to negative soil N₂O emissions [9]. Paddies are artificial wetlands that experience flooding continuously, with the free water layer above the soil surface and the oversaturated soil hindering the migration and diffusion of N₂O gas in the soil, which increases the probability of N₂O being reduced to N₂ [10,11]. Therefore, it is of great significance to study the N₂O consumption in flooded paddy soils for the control of global greenhouse gas emission.

The N₂O produced mainly by microbial nitrification and denitrification is trapped partly in soil water and soil pores when it migrates and diffuses in the soil profile, and part of it is converted into nitrogen (N₂) by *nosZ*-gene containing microorganisms, which is the effective N₂O consumption pathway [12]. Many abiotic factors influence denitrification rates, such as soil texture [13,14], soil pH [15,16], organic carbon content [17,18],

nitrogen content [11,19], etc. These factors also influence the structure and function of denitrifying microorganisms, which, in turn, affects N_2O consumption. Soil organic carbon can stimulate the respiration of soil microorganisms, create a hypoxic or even anaerobic environment for denitrifying microorganisms, and provide a favorable environment for the complete reduction of N_2O in soil to N_2 [20,21]. Soil pH indirectly affects the uptake and consumption of N_2O by affecting the activity of N_2O reductase [16,22]. Liu et al. [16] found a negative correlation between soil pH value (average 5.35 ± 0.54) and N_2O reduction rate in subtropical regions because lower soil pH values inhibit the activity of N_2O reductase and hinder the reduction of N_2O to N_2 . Saleh-Lakha et al. [22] also found that the activity of N_2O reductase decreases when $\text{pH} < 5.0$, while the activity of N_2O reductase is significantly enhanced at a higher pH, promoting the reduction of N_2O to N_2 . There are great differences in soil texture developed by different parent materials. Soil texture is an important factor affecting N_2O consumption because the air permeability is significantly weakened with the decrease in soil particle size, which is easier to form an anoxic environment and conducive to the N_2O consumption by denitrifying microorganisms [23].

Although more and more studies have focused on the N_2O consumption in different soil ecosystems, such as agroecosystems, forest ecosystems, estuary intertidal ecosystem, and so on [24–28], studies on the comparison of N_2O consumption in paddy soils developed from different parent materials are still lacking. Therefore, this study investigated nine paddy soils developed from three parent materials in subtropical China, setting two exogenous gas treatments (N_2O and He) for incubation in an anoxic and N_2 -free incubation system. The hypothesis of this study was that the N_2O consumption and N_2 production of flooded paddy soils developed from different parent materials are strong, but their dominant impact factors might be varied. Therefore, the objectives of this study were as follows: (1) quantifying the reduction of N_2O after flowing through a 5 cm depth soil layer to determine the N_2O consumption capacity of the paddy soil under flooded and anaerobic conditions; (2) quantifying the N_2 increment in this anoxic incubation system so as to elucidate the contribution of the N_2O microbial reduction process to the soil N_2O consumption capacity of nine paddy soils; (3) determining the correlation between the soil N_2O consumption capacity of three types of paddy soils and their related soil factors.

2. Materials and Methods

2.1. Soil Collection and Soil Physicochemical Properties

In this study, soils derived from quaternary red clay (Huanghua Town, Hubei Province, China, $28^\circ 17' 52''$ N and $113^\circ 15' 32''$ E), from lake sediment sand (Jinjing Town, Hubei Province, China, $28^\circ 33' 08''$ N and $113^\circ 24' 57''$ E), and from alluvial soil (Yueyang County, Hubei Province, China, $29^\circ 04' 55''$ N and $113^\circ 01' 06''$ E) were selected as the research objects, which were named R, S, C soils, respectively. These three types of paddy soils are widely distributed in the subtropical region of China.

In each sampling plot, three paddy sites were randomly selected for soil collection (nine sites in total), ensuring the same rice planting system and farm management. The distance between each site was greater than 5 km. The soil samples were collected by a five-point sampling method at a depth of 0–5 cm from each of the nine sites in February 2017 [29], named R1, R2, R3, S1, S2, S3, C1, C2, and C3, respectively. The soil samples from each site were passed through a 2 mm sieve, and visible plant residues were removed. Thereafter, the soil samples were air-dried and stored at room temperature until further use.

The initial properties of the nine soils are shown in Table 1. According to the international soil texture classification standard, R1, R2, and R3 were classified as loamy clay soil. S1, S2, and S3 were classified as sandy loam with almost 0% clay content and more than 75% sand and gravel content. C1, C2, and C3 were classified as silty clay loam.

Table 1. The properties of nine paddy soils developed from three parent materials.

Soil Types	Soil Texture			pH (H ₂ O)	SOC (%)	OM (%)	DOC (mg kg ^{−1})	AK (mg kg ^{−1})	TN (mg kg ^{−1})	NH ₄ ⁺ -N (mg kg ^{−1})	NO ₃ [−] -N (mg kg ^{−1})
	Sand %	Silt %	Clay %								
R1	46.31 ± 0.52 c	42.78 ± 0.52 c	10.91 ± 0.00 d	5.13 ± 0.03 d	1.66 ± 0.05 f	2.87 ± 0.08 f	295.13 ± 13.19 d	234.76 ± 2.29 a	1.64 ± 0.01 e	38.80 ± 0.68 d	7.32 ± 0.28 b
R2	44.56 ± 0.08 d	38.38 ± 0.08 d	17.06 ± 0.00 b	4.69 ± 0.02 f	1.67 ± 0.07 f	2.88 ± 0.11 f	343.41 ± 3.13 c	145.45 ± 4.20 c	1.52 ± 0.00 f	21.39 ± 0.52 f	2.58 ± 0.03 d
R3	42.01 ± 0.63 e	42.99 ± 0.63 c	15.00 ± 0.00 c	5.11 ± 0.01 d	1.97 ± 0.02 e	3.40 ± 0.04 e	338.78 ± 3.29 c	182.65 ± 1.97 b	2.03 ± 0.03 d	138.84 ± 0.34 a	4.19 ± 0.38 c
S1	75.81 ± 0.72 b	24.19 ± 0.72 e	0.00 ± 0.00 f	5.13 ± 0.06 d	3.13 ± 0.01 a	5.39 ± 0.02 a	401.25 ± 29.79 b	88.49 ± 2.02 ef	2.69 ± 0.07 a	41.40 ± 1.82 c	2.49 ± 0.29 d
S2	81.71 ± 0.12 a	18.29 ± 0.12 g	0.00 ± 0.00 f	4.84 ± 0.05 e	2.48 ± 0.04 c	4.28 ± 0.06 c	225.49 ± 7.56 e	48.98 ± 2.85 g	2.23 ± 0.04 c	20.39 ± 0.39 f	2.59 ± 0.55 d
S3	76.54 ± 0.21 b	22.74 ± 0.21 f	0.71 ± 0.00 e	4.85 ± 0.02 e	2.83 ± 0.12 b	4.88 ± 0.21 b	596.42 ± 22.09 a	51.18 ± 2.60 g	2.48 ± 0.16 b	92.90 ± 4.03 b	4.18 ± 0.28 c
C1	31.33 ± 1.37 f	51.60 ± 1.37 b	17.07 ± 0.01 b	6.16 ± 0.02 a	2.46 ± 0.06 c	4.24 ± 0.10 c	222.74 ± 5.02 e	123.00 ± 1.35 d	2.50 ± 0.01 b	26.25 ± 0.04 e	3.66 ± 0.16 c
C2	27.65 ± 0.80 g	55.31 ± 0.85 a	17.03 ± 0.09 b	5.33 ± 0.04 c	2.17 ± 0.05 d	3.75 ± 0.09 d	191.84 ± 1.31 f	91.61 ± 7.70 e	1.94 ± 0.00 d	20.90 ± 0.18 f	2.38 ± 0.22 d
C3	26.85 ± 0.11 g	50.68 ± 1.07 b	22.47 ± 1.10 a	6.09 ± 0.01 b	1.60 ± 0.04 f	2.76 ± 0.07 f	217.68 ± 9.82 e	83.23 ± 0.20 f	1.40 ± 0.01 g	15.29 ± 0.17 g	9.85 ± 0.88 a

Different lowercase letters within each column mean the differences between soil types are significant at the 0.05 level.

2.2. Experimental Design

Soil incubation device (Figure 1a): Each soil incubation device was constructed from a polyvinyl chloride (PVC) cylinder (height: 15.0 cm, inner diameter 8.5 cm, and outer diameter: 9.0 cm) with a screw sealing cover. A 2 mm diameter hole was drilled in the cylinder wall 1.5 cm above the bottom of the cylinder, and a second hole (5 mm in diameter) was drilled in the middle of the sealing cover. A three-way valve for gas sampling was connected to each hole. A silicone tube (length: 50 cm, inner diameter: 1.0 cm, and outer diameter: 1.4 cm) was sealed by inserting a rubber plug at each end, creating a disc shape. This silicone disc was positioned at the bottom of the PVC cylinder. Thereafter, a needle connected to the three-way valve was threaded into one end of the silicone tube through the hole in the cylinder wall, and the hole was sealed using glue.

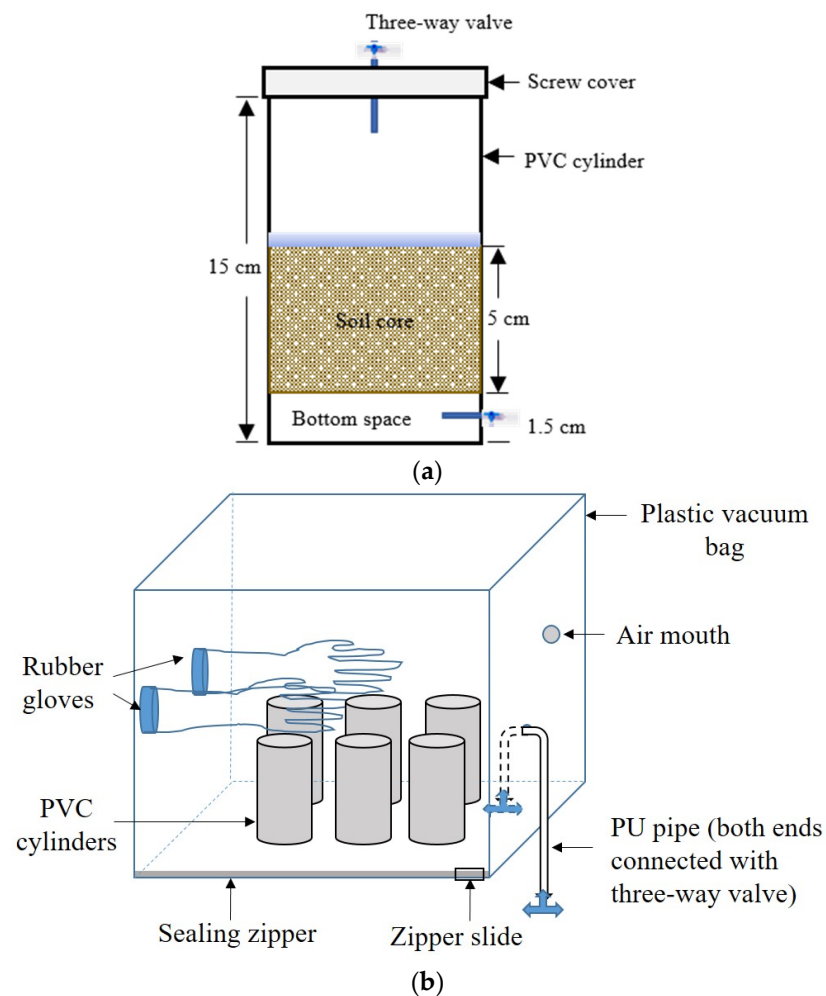


Figure 1. (a) Schematic diagram of the soil incubation device. The bottom space consisted of a disc-shaped sealed silicone tube placed at the bottom of the PVC cylinder and was used to add N₂O or He gas. (b) Outline of N₂-free microcosm system [29].

Soil pre-incubation: In each cylinder, 200 g of air-dried soil was packed above the silicone disc to a depth of 5 cm (the soil core in Figure 1a). Thereafter, 120 mL of deionized water was added to the soil surface to achieve a mass water content of 60% and a free water layer with a final depth of 0.5 cm. The cylinders were randomly left to pre-culture for one week, after which the soil moisture was completely balanced and stable, and the vitality of the soil microorganisms was restored [29].

Gas addition treatments: In this study, two gas addition treatments were applied to each soil: (1) N₂O was added to create the N₂O treatment, and (2) He was added to

create the CK treatment. Each treatment had three replicates; therefore, nine soils had 54 incubation devices in total.

Gas addition procedure: For adding N_2O or He gas into the bottom of soil cores, each PVC cylinder needed to be sealed completely using the screw covers and three-way valves, and the sealing effect was checked using the drainage method. Then, 30 mL of gas in the headspace was extracted through the three-way valve in the screw cover using a syringe; thereafter, the same amount of He was added to the headspace. This process was repeated five times to ensure that the air inside the PVC cylinder was completely replaced by pure He. And then 3 mL of N_2O (3401 $\mu\text{g N}_2\text{O-N}$) and 3 mL of He were injected into the bottom silicon tubes to start the incubation. All devices were incubated at 28 °C for 96 h.

2.3. Gas Collection and Measurement

The concentration of N_2O and N_2 in gas samples was accurately collected and measured by a N_2 -free microcosm system [29], which was not affected by a high concentration of N_2 in the atmosphere. It consists of a sealed vacuum bag with operating gloves and a polyurethane (PU) gas exchange tube (Figure 1b). Due to the limited space of a N_2 -free microcosm system, the PVC cylinders of the paddy soil developed from the same parent material, including N_2O treatment and CK treatment, were put into a set of N_2 -free microcosm systems to ensure that the differences between treatments were minimized. In total, three sets of the N_2 -free microcosm system were used at the same time. All PVC cylinders were put into the corresponding vacuum bag of the N_2 -free microcosm system, as well as gas sampling bottles and syringes. After the vacuum bags were sealed, pure helium gas was replaced with the bag's air through the PU tube more than three times to ensure that there was no N_2 in the vacuum bags [29]. Gas collection was performed in the N_2 -free microcosm system by operating gloves.

After the addition of the 3 mL of N_2O and He gas to the bottom silicon tubes, 20 mL of gas was collected through the three-way valve in the screw cover of each incubation device as the 0 h gas sample from the headspace. Thereafter, 20 mL of gas was collected from the headspace after 2, 5, 8, 11, 14, 23, 30, 37, 47, 54, 61, 71, and 96 h of incubation, which were chosen through a preliminary experiment to reflect the key turning points for the increase and decrease in the N_2O concentration. Before each gas sample collection, we used a syringe to repeatedly pump the gas in the upper space of the jar to mix the gas in the device. In addition, 5 mL of the bottom space gas was collected from the bottom silicon tube at 96 h. The gas volume in each glass sampling bottle was filled up to 20 mL with pure He for the determination of N_2O concentration ($\mu\text{mol}\cdot\text{mol}^{-1}$). The N_2O concentration in each gas sample was determined using a gas chromatograph (Agilent 7890A; Agilent Technologies, Inc., Wilmington, DE, USA).

For N_2 determination, 20 mL of gas samples was collected from the headspace of each device at 0, 5, 11, 23, 30, 37, 47, 61, 71, and 96 h of incubation. The N_2 concentration (%) of the gas samples collected during this experiment was determined using a gas chromatograph (Agilent 7890A; Agilent Technologies, Inc., Wilmington, DE, USA) equipped with a self-made anaerobic incubator. The injection volume of the gas chromatograph was 250 μL ; a 5 Å molecular sieve column (3 m $\times$ 1/8") linked with a packed column (PorapakQ, 6ft) was heated to a temperature of 100 °C; the constant current amount of the production electron current (EPC) was 8 mL/min, and the analysis time was 2 min [30].

2.4. Soil Sampling and Measurement

During the incubation period, soil samples were collected from each incubation device at 0 h and 96 h to evaluate the consumption of soil ammonium–nitrogen ($\text{NH}_4^+\text{-N}$), nitrate–nitrogen ($\text{NO}_3^-\text{-N}$), and dissolved organic carbon (DOC) contents. The 100 g soil samples were collected from each incubation device (54 samples in total), and the core of the 0–5 cm deep soil was collected using a sterilized, long-handled spoon. Because the sampling procedure was destructive, the sampled incubation devices were discarded after soil sampling. To facilitate the soil sampling process, the free water layer in each incubation

device was drained, and the soil samples were collected immediately after the surface water had been removed. After fully mixing the soil samples in each incubation device, they were stored at -4°C for the determination of the soil properties. The 50 mL of soil extracts in 0.5 M K_2SO_4 (1:10, *w:v*) from 10 g of fresh soil was used to analyze the soil $\text{NO}_3^- - \text{N}$ and $\text{NH}_4^+ - \text{N}$ by a continuous flow analyzer (FIA star 5000, Foss, Gothenburg, Sweden), and the soil DOC was analyzed by a C/N analyzer (TOC-VWS, Shimadzu, Kyoto, Japan). Soil pH was determined by a pH meter (FE-20, METTLER TOLEDO, Shanghai, China) in the suspension of 1 M KCL (dry soil: solution = 1:5, *w/v*) [11]. Soil texture was measured by a fixed pipette method, soil organic carbon (SOC) was determined by oxidation with potassium dichromate in concentrated H_2SO_4 , and the available potassium (AK) and total nitrogen (TN) of the air-dried soils were determined by Atomic Absorption Spectrometer (ICE3000, Thermo Fisher, Waltham, MA, USA) using the methods described by Xing et al. [13].

2.5. Calculations

The mass (mg) of N_2O or N_2 in the gas samples was calculated using Formula (1):

$$m = CMV/Vm \quad (1)$$

where C ($\mu\text{mol}\cdot\text{mol}^{-1}$ for N_2O , % for N_2) is the gas concentration directly determined by the gas chromatograph, M ($\text{g}\cdot\text{mol}^{-1}$) is the relative molecular weight of the gas, V (L) is the gas volume for a 31.4 mL bottom space and 453.73 mL headspace volume, and Vm is the standard gas molar mass of $22.4\text{ L}\cdot\text{mol}^{-1}$.

The N_2 total increment was calculated using Formula (2):

$$\text{Increment}(\text{N}_2) = (m\text{N}_2\text{O}(96\text{ h}) - m\text{N}_2\text{O}(0\text{ h})) - (m\text{CK}(96\text{ h}) - m\text{CK}(0\text{ h})) \quad (2)$$

where $m\text{N}_2\text{O}(96\text{ h})$ is the N_2 amount determined from the N_2O treatment at 96 h, and $m\text{CK}(0\text{ h})$ is the N_2 amount determined from the CK treatment at 0 h. The N_2O consumption during the entire incubation was calculated as the N_2O addition at 0 h minus the N_2O amount after 96 h of incubation.

2.6. Statistical Analysis

After determining the distribution of the data and transforming the non-normal data, one-way analysis of variance (ANOVA) was used to show the differences in the amounts of N_2O and N_2 , as well as the soil nutrient consumption for the two gas treatments and nine soil types. Multivariate analysis of variance was used to determine the effects of gas extraction time, treatment level, and soil type on the changes in N_2O and N_2 concentrations in the upper space. The post hoc test was performed using the LSD test. Principal component analysis (PCA) was performed on the relationship between soil N_2O consumption and soil factors, and linear regression analysis was performed on the relationship between major soil factors and soil N_2O consumption; the significance test was conducted using F test. Significance was accepted at a level of probability of $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics 25 (SPSS Inc., Chicago, IL, USA), and graphs were drawn using Origin 2019b version (OriginLab, Northampton, MA, USA).

3. Results

3.1. Cumulative Concentrations Changes of N_2O and N_2 Above the Soil Cores

The N_2O concentration in the headspace of nine paddy soils under CK treatment remained at a low level during the entire incubation period, ranging from 0 to $1.38\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$ (Figure 2). The N_2O concentration above nine soil cores under N_2O treatment showed a significant increase ($p < 0.001$, Table 2), with different peak values, and then gradually decreased. Soils R2 and S3 reached a peak emission at 71 h (Figure 2b,f), with 159.58 and $51.3\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$, respectively, and then N_2O emissions decreased, but the accumu-

lated N_2O concentration at 96 h was still at a high level (141.06 and $31.93 \mu\text{mol mol}^{-1}$). In addition, the N_2O concentration of all other soils decreased to the CK level after 96 h of incubation. The cumulative N_2O emissions of soils C1 and C3 remained at a low level during the incubation period (Figure 2g,i), while soil C2 reached a peak emission of $2.26 \mu\text{mol mol}^{-1}$ at 30 h (Figure 2h). Multivariate analysis of variance showed that time, treatment, and soil type and their interactions all significantly affected the cumulative concentration of N_2O above the soil cores ($p < 0.01$, Table 2).

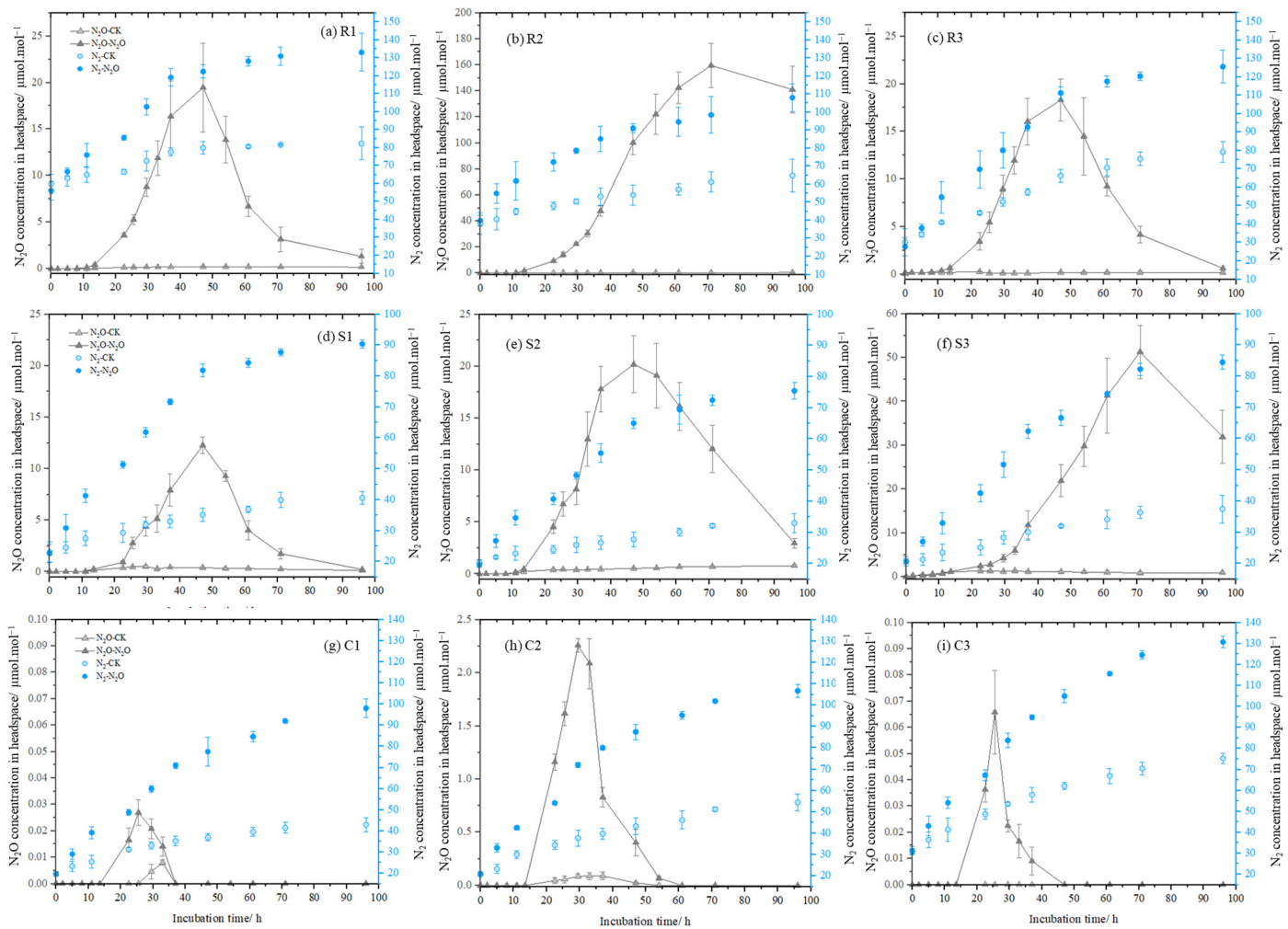


Figure 2. Dynamics of the cumulative concentrations of N_2O and N_2 in the headspace above the surface of three loam paddy soils (a–c), three sandy loam paddy soils (d–f), and three silt clay loam paddy soils (g–i) in incubation devices. The vertical bars indicate the standard error of the mean ($n = 3$).

Table 2. Multivariate analysis of variance of N_2O and N_2 concentrations.

	Times	Treatments	Soil Types	Times $\times$ Treatments	Times $\times$ Soil Types	Treatments $\times$ Soil Types	Treatments $\times$ Times $\times$ Soil Types
N_2O	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
N_2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.s

Times: Gas sampling time. Treatments: N_2O and CK treatments. n.s = not significant ($p < 0.05$, LSD text).

During the 96 h flooded incubation, the N_2 concentrations above the nine paddy soil cores treated with CK increased gradually and reached the maximum value at 96 h. The variation tendency of the N_2 concentration in the headspace treated with N_2O addition with

time was in consistency with that the CK, but the increasing range significantly exceeded their CK treatment ($p < 0.05$, Figure 2). And the difference in N_2 concentration in the headspace of the N_2O treatment and CK treatment varied among nine paddy soils. Time, treatment, and soil type significantly affected the cumulative concentration of N_2 in the upper space ($p < 0.01$), but the interaction of the three had no significant effect on the cumulative concentration of N_2 (Table 2).

3.2. Total N_2O Consumption and N_2 Increment

Table 3 shows that the N_2 content at 0 h for both the N_2O treatment and CK treatment was not 0 mg, indicating that the replacement of He in the incubation devices or N_2 -free incubation system did not reach 100%. However, both treatments for paddy soil developed from same parent material were placed in the same N_2 -free incubation system, and the ANOVA results suggested there was no significant difference in the N_2 content at 0 h between the two gas treatments for each soil ($p > 0.05$).

Table 3. The total N_2O consumption and N_2 increment of nine paddy soils during 96 h incubation period.

Soil Types	N_2O Addition/ μ g	N_2O Content in Pot/ μ g		N_2O Total Consumption/ μ g	N_2 Content of N_2O Treatment in Pot/mg		N_2 Content of CK Treatment in Pot/mg		N_2 Total Increment/mg
		0 h	96 h		0 h	96 h	0 h	96 h	
R1	3401.16	0.02 ± 0.01 c	4.91 ± 0.56 d	3396.27 ± 0.56 ab	2.88 ± 0.28 a	6.84 ± 0.54 a	3.07 ± 0.27 a	4.23 ± 0.47 a	2.80 ± 0.25 a
R2		0.02 ± 0.00 c	78.04 ± 9.80 b	3323.14 ± 9.80 c	2.05 ± 0.16 b	5.56 ± 0.39 b	1.97 ± 0.31 b	3.33 ± 0.47 b	2.14 ± 0.07 d
R3		0.12 ± 0.00 a	4.68 ± 0.43 d	3396.60 ± 0.43 ab	1.43 ± 0.26 c	6.46 ± 0.46 a	1.55 ± 0.38 d	4.08 ± 0.29 a	2.51 ± 0.25 abcd
S1		0.01 ± 0.00 c	2.61 ± 0.67 f	3398.56 ± 0.67 a	1.16 ± 0.15 d	4.65 ± 0.07 cd	1.19 ± 0.17 e	2.09 ± 0.11 d	2.59 ± 0.19 abc
S2		nd	169.64 ± 22.51 a	3231.53 ± 22.51 d	1.01 ± 0.04 d	3.88 ± 0.13 e	1.05 ± 0.05 e	1.70 ± 0.16 d	2.22 ± 0.19 cd
S3		0.05 ± 0.01 b	18.53 ± 3.00 c	3382.68 ± 3.00 b	1.06 ± 0.07 d	4.35 ± 0.12 d	1.06 ± 0.04 e	1.92 ± 0.23 d	2.42 ± 0.22 bcd
C1		nd	1.69 ± 0.37 f	3399.47 ± 0.37 a	1.02 ± 0.03 d	5.04 ± 0.22 bc	1.01 ± 0.06 e	2.21 ± 0.17 d	2.82 ± 0.23 a
C2		nd	2.14 ± 0.05 f	3399.03 ± 0.05 a	1.08 ± 0.02 d	5.49 ± 0.15 b	1.05 ± 0.03 e	2.80 ± 0.20 c	2.66 ± 0.03 ab
C3		nd	2.49 ± 0.67 f	3398.67 ± 0.67 a	1.61 ± 0.11 c	6.73 ± 0.14 a	1.59 ± 0.07 d	3.87 ± 13 a	2.84 ± 0.21 a

nd means N_2O concentration is lower than the determination limit. Different lowercase letters within each column mean the differences between soil types are significant at the 0.05 level.

Over 95.01% of the N_2O added to the nine soils disappeared by the 96 h incubation, and the total N_2O consumption of the C1, C2, and C3 soils was almost equal (exceeding 99.93%), significantly higher than that of the R and S soils (Table 3). After 96 h of incubation, the N_2 content of the nine soils in the N_2O treatment significantly exceeded their CK treatment ($p < 0.05$). The N_2 total increments in the nine soils accounted for 64.49% ~ 83.65% of their total N_2O consumption, and three C soils had the highest N_2 total increments, with an average value of 2.77 mg N_2 -N.

3.3. Changes in Soil Inorganic Nitrogen and DOC

During the 96 h incubation period, the NH_4^+ -N content in all nine soils of the N_2O treatment and CK treatment increased (Figure 3a), and the NH_4^+ -N increment of the CK treatment was higher than its N_2O treatment. The difference in NH_4^+ -N increment between the N_2O treatment and CK treatment was highly significant in the R1, R3, and C2 soils ($p < 0.01$), and R3 had the largest NH_4^+ -N increment among the nine paddy soils. Soil NO_3^- -N content was consumed by flooding paddy soils during incubation, except the S1 soil, but the consumption of NO_3^- -N content was lower than 0.85 mg/kg (Figure 3b). After 96 h of incubation, the DOC content in the nine soils was all consumed, with about 25.30~40.89% in the N_2O treatment and 5.79~34.42% in the CK treatment, respectively (Figure 3c). And the difference in DOC consumption between the N_2O treatment and CK treatment was extremely significant in three R soils and the C2 soil ($p < 0.001$), and it was significant in the S1 and S2 soils.

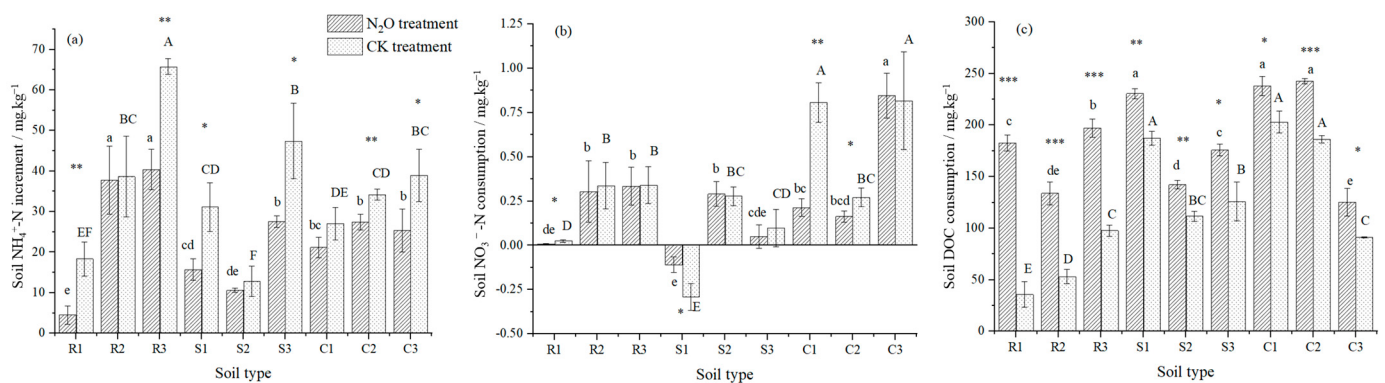


Figure 3. Changes in (a) soil $\text{NH}_4^+\text{-N}$ increment, (b) soil $\text{NO}_3^-\text{-N}$ consumption, and (c) soil DOC consumption of the R soils, S soils, and C soils during the 96 h incubation. The vertical bars indicate the standard error of the mean ($n = 3$). Different lowercase letters indicate significant differences among nine soils in the N_2O treatment ($p < 0.05$), and uppercase letters indicate significant differences among nine soils in the CK treatment ($p < 0.05$). *, **, and *** indicate significant differences at the 0.05, 0.01, and 0.001 levels between the N_2O treatment and CK treatment of each soil.

3.4. Correlation Between N_2O Consumption and Soil Factors in Different Types of Paddy Soil

The results of PCA analysis (Figure 4a) showed that there were significant clusters (95% confidence) in the N_2O consumption of three types of soil, and the dominant soil factors affecting N_2O consumption in the R soils, S soils, and C soils were varied. For three S soils, the main soil factor related to N_2O consumption was soil sand content, and there was a significant linear correlation between them ($R^2 = 0.95$, $p < 0.001$, Figure 4b). Soil available potassium (AK) content was the main soil factor regulating N_2O consumption in three R soils ($R^2 = 0.60$, $p < 0.01$, Figure 4c). And for C soils with the strongest N_2O consumption capacity, many soil factors, like soil clay content, silt content, and pH, were related to their N_2O consumption, but only soil clay content was significantly correlated with N_2O consumption at the 0.05 level.

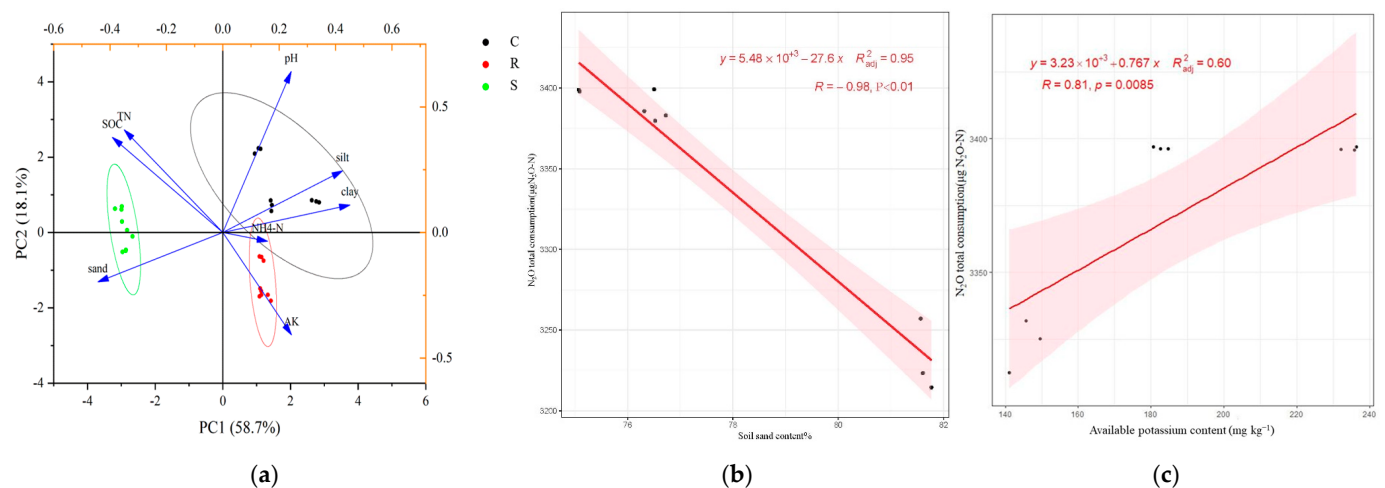


Figure 4. (a) PCA of N_2O consumption in loam paddy soils (R), sandy loam paddy soils (S), and silt clay loam paddy soils (C) with various soil factors; (b) regression analysis of total N_2O consumption and soil sand content in S soils, and (c) total N_2O consumption and available potassium in R soils.

4. Discussion

4.1. N_2O Consumption Potential of Flooded Paddy Soils Was Considerable

Paddy soils are an important source of N_2O emissions, and numerous studies have shown that the alternation of wet and dry processes in paddy soils can lead to large amounts

of N_2O emissions [31,32]. However, due to the special water demand of rice, paddy soils need to be kept flooded at the heading stage. Long-term flooding management prolongs the retention time of N_2O in the soil, which increases the probability of N_2O in the soil being reduced to N_2 and greatly reduces the net N_2O emissions [8,10,33]. The nine paddy soils in this study were all flooded (water content by mass, 60%). A large amount of N_2O passing through the bottom of the flooded soil body was trapped and consumed by the 5 cm soil layer, and only 0.05–4.99% of the N_2O was discharged to the surface, implying that paddy surface soils could reduce the net N_2O emission by more than 95.01% in a flooded and anaerobic state. Numerous studies have also reported the inconsistency between N_2O accumulation in soil profiles and surface N_2O emissions [11,34,35]. For instance, Wen et al. [34] monitored N_2O fluxes in cropland soil by measuring soil core airflow and found that the net flux of N_2O in the cropland soil accounted for about 24% of the total N_2O production, and voluminous N_2O was reduced to N_2 by denitrification. Additionally, Gao et al. [35] showed that the proportion of soil N_2O consumption in the intertidal soils of the Yangtze River Estuary was as high as 69.56–90.31%, and further research indicated that the N_2O consumption proportion generally increased from high (winter: $94.1 \pm 4.30\%$; summer: $96.5 \pm 1.51\%$) to low tidal zones (winter: $99.5 \pm 0.14\%$; summer: $99.7 \pm 0.13\%$).

The N_2O consumption process in soil can be divided into an abiotic-involved N_2O interception process, which mainly includes the dissolution of N_2O in soil water and the immobilization in soil pores [10,24], and a microbial N_2O reduction process [26]. Compared with other ambient gases, N_2O has a high solubility in water, up to $5.9 \text{ mL} \cdot \text{L}^{-1}$ at 25°C and 101.3 kPa [25,36]. In this study, each soil moisture content was set to a 60% mass moisture content, the soil pores were completely filled with water, and the amount of soluble N_2O was $139.07 \text{ } \mu\text{g}$, accounting for 4.09–4.3% of the total soil N_2O consumption. Additionally, the anaerobic environment formed by a higher soil pore water content can increase the activity of N_2O reductase, which is conducive to the complete denitrification process, and it promotes the procession of soil N_2O to reduce to N_2 , reducing N_2O emissions [37]. In addition, abundant organic carbon content and extremely low nitrate (NO_3^-) content are also considered to be favorable conditions for denitrification [38,39]. Huang et al. [40] indicated that adding glucose to soil can increase microbial activity, thereby increasing N_2O consumption. Murray et al. [41] showed that a low soil NO_3^- content is beneficial to N_2O consumption, while increasing soil NO_3^- content can inhibit the activity of N_2O reductase, thereby increasing the ratio of $\text{N}_2\text{O}/(\text{N}_2 + \text{N}_2\text{O})$. In this study, although no additional DOC was added, the soil background content of DOC was at a high level, reaching a maximum of $596.42 \text{ mg} \cdot \text{kg}^{-1}$. The DOC content of the nine soils decreased significantly after the incubation, and the NO_3^- contents were all at a low level in the nine soils ($<10 \text{ mg} \cdot \text{kg}^{-1}$), thus promoting the reduction of soil N_2O .

In addition, soil also absorbs and consumes N_2O from the atmosphere [34,42]. As the International Atomic Energy Agency (IAEA, 1992) estimates, the global soil N_2O uptake consumption was $7\text{--}13 \text{ Tg } \text{N}_2\text{O-N } \text{y}^{-1}$, the same via photolysis in the stratosphere ($9 \text{ Tg } \text{N}_2\text{O-N } \text{y}^{-1}$) [43]. In an Intergovernmental Panel on Climate Change (IPCC) work report, soil N_2O uptake was listed for the first time as an important potential N_2O sink in the world, and studies have begun to focus on soil N_2O uptake and consumption capacity [44]. Our results showed that the net accumulation of N_2O on the soil surface in the nine paddy soils showed a unimodal curve (there was also a continuous N_2O reduction trend after the emission peak), which meant that there was a net uptake of N_2O , and the uptake rate ranged from 13.22 to $140.33 \text{ } \mu\text{g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$. Chapuis-Lardy et al. [7] summarized that many types of soils can absorb N_2O from the atmosphere, and the total N_2O absorption rate was $0.0014\text{--}484 \text{ } \mu\text{g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$. Among them, the higher N_2O uptake potential is mainly concentrated in saturated soils such as peatlands, wetlands, and paddy fields, and the N_2O uptake rate reached $100\text{--}207 \text{ } \mu\text{g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ [45]. The previous study showed that the uptake rate of N_2O in a water-saturated paddy soil was $1.24\text{--}4667.99 \text{ } \mu\text{g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ [46]. The N_2O uptake rate in this study indicates that there is potential for N_2O uptake in paddy fields,

which can act as an important sink for atmospheric N_2O . Therefore, it is necessary to study the uptake of N_2O by soil to reduce atmospheric N_2O emission.

4.2. The Impact Factors Dominating the N_2O Consumption and N_2 Production of Flooded Paddy Soils Vary

The accumulation dynamics of N_2 in nine paddy soils were monitored, and they showed that there were significant differences in the dynamics of N_2 production and its proportion to total N_2O consumption in different paddy soils ($p < 0.05$). The differences in N_2 production indicated that the denitrifying microbial intensities in the nine paddy soils may be significantly different in the condition of different soil environmental factors. The reduction of N_2O to N_2 by soil denitrifying microorganisms is the only effective pathway for N_2O consumption in flooded and anaerobic paddy soils [38,47]. There are great differences in soil texture developed from different parent materials. Soil texture affects soil permeability, porosity, and water retention, which, in turn, results in differences in the types and quantities of denitrifying microorganisms, resulting in differences in N_2O consumption capacity [23,48]. The results of PCA showed that the dominant factor of N_2O consumption in three S soils was the soil sand content, and its N_2O consumption decreased linearly with the increase in the sand content (Figure 4b). This is because the sand content is high (Table 1), the soil is loose, and the gas permeability is good; thus, the gas diffusion speed is fast, which is not conducive to the formation of a N_2O reduction environment [14].

Soil silt, clay content, and soil pH were the related factors of N_2O consumption in C soils, and soil clay content was positively correlated with N_2O consumption (Figure 4a). The main reason is that the silty loam is heavier in texture, has strong water retention capacity, easily retains N_2O in the soil, and it is more likely to form an anoxic environment, which is conducive to the growth of denitrifying microorganisms [23,27]. Włodarczyk et al. [23] reported that the N_2O consumption of loam and silty loam accounted for 99.2% and 100% of the N_2O production, which was significantly higher than that of sandy soil during the 34 days of incubation at 20 °C. Soil pH can alter soil microbial community composition and affect N_2O reductase activity [12]. A previous study tested the effect of spatial variation in soil pH on off-season N_2O emission in an agricultural soil, and the results showed that the ratio of soil $\text{N}_2\text{O}/\text{N}_2$ decreases exponentially with increasing soil pH [49]. The dominant factors of N_2O consumption in R soils are soil available potassium content (AK) and $\text{NH}_4^+\text{-N}$ increasing content, because soil $\text{NH}_4^+\text{-N}$ provides nutrients and energy required for microbial activities, thereby promoting the process of N_2O reduction to N_2 [19]. Zhang et al. [50] showed that $\text{NH}_4^+\text{-N}$ was positively correlated with the abundance of denitrifying microbial communities. Soil AK content also has an impact on N_2O -reducing microorganisms. Previous studies have indicated that AK is significantly positively correlated with the abundance of denitrifying bacterial communities [51]. Therefore, the soil factors that cause differences in soil N_2O consumption are complex, and there are differences in the main soil factors affecting N_2O consumption in different soils.

5. Conclusions

Our results showed that more than 95.01% of the N_2O in the soil profile could be consumed in the surface of the paddy soils at a depth of 0–5 cm under flooding and anaerobic conditions, and most of it was reduced to N_2 by microorganisms, accounting for 64.49–83.65% of the total N_2O consumption. The N_2O consumption capacity and N_2 increment of C soils were significantly stronger than those of R soils and S soils. After the cultivation, soil DOC dropped significantly, indicating that microorganisms consuming DOC made a great contribution to the reduction of soil N_2O . In addition, there were significant differences in the N_2O consumption capacity in paddy soils developed from three parent materials. The N_2O consumption of S soils was mainly regulated by soil sand content, and the N_2O consumption of R soils was significantly affected by soil available potassium content. For C soils, more soil factors were related to their N_2O consumption. These results suggest that flooded paddy soils can consume large amounts of N_2O produced

in soil profiles regardless of the parent material. In addition, we can find the dominant soil factors affecting N_2O consumption in each type of paddy soil and regulate them to promote N_2O consumption and then reduce N_2O emission; for example, adding soil potassium fertilizer in paddy soils developed from quaternary red clay or adjusting soil texture in paddy soils developed from lake sediment sand to regulate N_2O emission from rice fields.

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