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Isotopic Signatures and Concentration Profiles of Nitrous Oxide in a Rice-Based Ecosystem During the Drained Crop-Growing Season

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Isotopic signatures and concentration profiles of nitrous oxide in a rice-based ecosystem during the drained crop-growing season

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[1] The stable isotopic composition of nitrous oxide (N_2O) in agricultural soils can improve our understanding of the relative contributions of the main microbial processes (nitrification and denitrification) responsible for N_2O formation in soils, and can provide constraints for the atmospheric N_2O budget. Here, we present soil profiles featuring N_2O concentrations and $\delta^{15}N$ and $\delta^{18}O$ values in N_2O over time, which permit the in situ identification of processes and sites of N_2O production in a rice-based ecosystem seeded with winter wheat. Our $\delta^{15}N$ and $\delta^{18}O$ soil profile values support the conclusion that denitrification is the dominant process behind N_2O production during the winter wheat season. The soil N_2O gas concentrations are higher below 10 cm than above 10 cm, and more depleted in ^{15}N-N_2O at 10−15 cm depth compared to other soil depths, which indicates that the N_2O production zone is located at a depth of 10−15 cm within 0−20 cm. The $\delta^{15}N_{air}$ and $\delta^{18}O_{SMOW}$ ($\delta^{18}O$ of standard mean ocean water) values for soil gas N_2O averaged over the entire wheat growing season are $+0.90 \pm 2.9$ ($n = 64$) and $+39.3 \pm 3.1$ ($n = 64$), respectively. Given that paddy source N_2O is much heavier in both measured ^{15}N and ^{18}O compared to other fertilized soils, agricultural soils have been underestimated as a source of N_2O since isotopic constraints from rice agriculture have not been taken into account.

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1. Introduction

[2] There is growing concern about increasing emissions of greenhouse and ozone-depleting gases such as nitrous oxide (N_2O) [*Intergovernmental Panel on Climate Change* (*IPCC*), 2007]. Agricultural soil is the largest anthropogenic source of N_2O to the troposphere [*Kroeze et al.*, 1999; *IPCC*, 2007]. Rice paddies comprise a significant fraction of the world's agricultural lands, and the majority of rice paddies in China are cultivated using a flooded rice-drained crop system that rotates crops on an annual basis [*Xiong et al.*, 2008]. Rice paddies are a significant source of atmospheric N_2O, which is emitted primarily when paddies are drained during rice-growing and non-rice-growing seasons [*Cai et al.*, 1997; *Xing et al.*, 2002b; *Khalil and Shearer*, 2006]. The observed N_2O flux is generally assumed to be derived from microbial processes in the topsoil layer where most applied mineral N persists [*Granli and Bøckman*, 1994], though subsoil denitrification with N_2O production and entrapment has been identified as a process potentially capable of significant N_2O production rates [*Clough et al.*, 2001; *Well et al.*, 2001; *Xing et al.*, 2002a; *Xiong et al.*, 2006]. Given the unique structure of a paddy field, with its ploughed layer and compacted foundation, it is necessary to specifically locate the N_2O production region within the soil profile.

[3] Microbial transformations of ammonium (NH_4^+) and nitrate (NO_3^-) [*Bouwman*, 1998] are considered the main processes producing N_2O in terrestrial ecosystems, in particular nitrification, denitrification, chemodenitrification [*van Cleemput*, 1998], and fungal transformations [*Shoun et al.*, 1992; *Laughlin and Stevens*, 2002]. The microorganisms responsible operate under various conditions, with nitrification being the predominant N_2O-producing process in moderately moist, warm, and NH_4^+-rich soils. In contrast, denitrification is the predominant process under wet (anaerobic), NO_3-rich soil conditions [*Conrad*, 1996; *Bouwman*, 1998]. Neither mechanism is completely understood, which leads to uncertainties in quantifying N_2O emissions [*Prather and Ehhalt*, 2001; *Sowers et al.*, 2002; *Stein and Yung*, 2003]. This, in turn, makes it difficult to create an effective strategy for mitigating N_2O emissions.

[4] Stable isotopic signatures of N_2O have been used to study sink and source processes in terrestrial and aquatic systems, as well as in the atmosphere [*Stein and Yung*, 2003]. The stable isotope ratio of $^{15}N/^{14}N$ in denitrifier-derived N_2O can differ from that of nitrifier-derived N_2O [*Kim and Craig*, 1990; *Webster and Hopkins*, 1996]. The

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^{15}N enrichment factor (ε) values (defined as $\varepsilon = \delta^{15}N\text{-}N_2O_{emitted} - \delta^{15}N_{substrate}$) for nitrification and denitrification in pure bacterial cultures show more negative values for nitrifying bacteria (ε = −45‰ to −66‰) compared to denitrifying bacteria (ε = −12‰ to −28‰) [*Wahlen and Yoshinari*, 1985; *Yoshida*, 1988; *Webster and Hopkins*, 1996; *Yoshinari et al.*, 1997; *Barford et al.*, 1999]. ^{15}N-depleted N_2O found in aerobic aquifers and oceans has been attributed to nitrification [*Ueda et al.*, 1991]. The amount of ^{18}O in the N_2O emitted from soils also provides information on microbially produced N_2O. Further reduction of N_2O to N_2 during denitrification results in an enrichment of ^{15}N [*Barford et al.*, 1999; *Boontanon et al.*, 2000; *Mandernack et al.*, 2000; *Vieten et al.*, 2007] and ^{18}O [*Yoshinari et al.*, 1997; *Dore et al.*, 1998; *Naqvi et al.*, 1998; *Ostrom et al.*, 2000] in residual N_2O, which explains the enriched ^{15}N values of N_2O measured in lakes, oceans [*Naqvi et al.*, 1998], and soils [*Mandernack et al.*, 2000; *Tilsner et al.*, 2003; *Wrage et al.*, 2004]. In principle, this makes it possible to use the isotopic signature of emitted N_2O, in concert with N_2O concentration data, to identify either nitrification and denitrification as the main process behind N_2O production in soils.

[5] Stable isotopic signatures of N_2O have also been used to constrain atmospheric N_2O budgets [*Röckmann et al.*, 2003; *Sowers et al.*, 2002; *Bernard et al.*, 2006]. Large uncertainties in N_2O budgets revealed by models [*Sowers et al.*, 2002] stem from uncertainties in the isotopic signatures of various source components, particularly the soil source [*Yamulki et al.*, 2000; *Pérez et al.*, 2000, 2001, 2006; *Bol et al.*, 2003; *Wrage et al.*, 2004; *Ogawa and Yoshida*, 2005]. Little information on the isotopic signatures of N_2O from rice-based ecosystems has been reported. Thus, additional characterization of the isotopic signatures of N_2O produced in soils is required to further constrain the global N_2O budget using stable isotopes.

[6] In light of this, the objectives of this study were (1) to isolate the production site for N_2O within 0–20 cm in paddy soils, (2) to identify the N_2O production processes of a paddy soil, and (3) to reduce uncertainties in the isotopic signatures of N_2O produced in rice paddies.

2. Materials and Methods

2.1. Experimental Setup

[7] Field studies were performed on a paddy field near Nanjing, China (31°58′N, 118°48′E). Like most paddy fields in China, this field has been subjected to annual rotations of a single flooded rice crop and a drained winter crop (winter wheat or oil-seed rape) for hundreds of years. The soil in this region is classified as *Stagnic Luvisols* with pH of 5.71, 14.7 g kg^{-1} organic C, and 1.32 g kg^{-1} total N.

[8] In 2005, 24 plots (each 4 m × 5 m) were established for this study. Following traditional management procedures, the plots were subjected to annual rotations of a single flooded rice crop and a drained crop of winter wheat. The first wheat crop grew between 16 November 2005 and 17 May 2006, and the second wheat crop grew between 6 November 2006 and 18 May 2007. During the rice-growing season preceding each wheat crop, eight treatments with an experimental design of 2^3 were managed in triplicate. This involved three factors with two different conditions explored for each factor: water management (midseason drainage with intermittent flooding versus continuous flooding), organic matter amendments (rice straw added versus no straw added), and nitrogen fertilization (N application versus no N application). All plots were drained for wheat sowing, received water via precipitation only, and all received the same rate of fertilizers during the wheat-growing season. N fertilizer was applied as urea at a total rate of 220 kg N ha^{-1} crop^{-1} in a ratio of 4:3:3 at three intervals (16 November 2005, 4 February 2006 and 16 March 2006 for the first wheat crop and on 6 November 2006, 19 January and 8 March 2007 for the second wheat crop). Phosphorous and potassium fertilizers were applied only for basal fertilization.

[9] A multilevel soil gas sampler was installed in each plot. This was used to collect soil gas from depths of 0–5, 5–10, 10–15, and 15–20 cm. The soil gas sampler was built using PVC pipes (i.d. Four cm), each with two rows of six contact holes (Φ1.5 cm) at the desired soil depth.

[10] Soil redox potential (Eh) and soil temperature (T) probes were installed in one plot of each treatment during the rice season. The soil Eh probe was made of platinum wire (calomel reference electrode) and the T probe was a Pt thermocouple (HEL-777-A, 1000 Ω). Each probe sensor was installed at the desired soil depth of 5 cm, 10 cm, 15 cm, or 20 cm. The system was field tested before rice transplanting in May 2005.

2.2. N_2O Flux and Soil Parameter Measurements

[11] N_2O flux measurements were combined with detailed measurements of soil NO_3- and NH_4^+ concentrations, as well as moisture content. N_2O emissions were quantified with a closed chamber technique [*Hutchinson and Mosier*, 1981]. Sampling chambers were made of with 60 × 70 × 50 cm Plexiglass (or 100 cm when plants are higher). Chambers were placed on a fixed PVC frame in each plot. Gas samples were usually collected in the morning with needle ends connected to evacuated vial bottles (18.5 mL) stoppered with butyl rubber septae. For every N_2O flux measurement, gas samples from each chamber were withdrawn at 15, 30, and 45 min intervals after closing the chamber. N_2O fluxes were examined at approximately 10-day intervals from 24 field plots during the first wheat-growing season from 18 November 2005 to 17 May 2006. Gas samples (both chamber flux and soil gas) were analyzed for N_2O on a gas chromatograph HP5980 equipped with an ECD and back flush controlled by a 10-port valve [*Xiong et al.*, 2002].

[12] Soil samples for monitoring soil nitrate, ammonium, and moisture were collected at depths of 0–15 cm from six points in each plot. Around 40 g of fresh soil from subsampling with a quartering method were extracted with 2 M KCL and used to analyze soil nitrate and ammonium. Soil moisture was measured by a gravimetric procedure following standard oven drying.

2.3. Soil N_2O Gas Profiles and Parameter Measurements

[13] The multilevel soil gas sampling probes (0–5 cm, 5–10 cm, 10–15 cm, and 15–20 cm) were designed to monitor soil gas N_2O concentration profiles for the 24 plots simultaneously with N_2O flux measurements. However, we

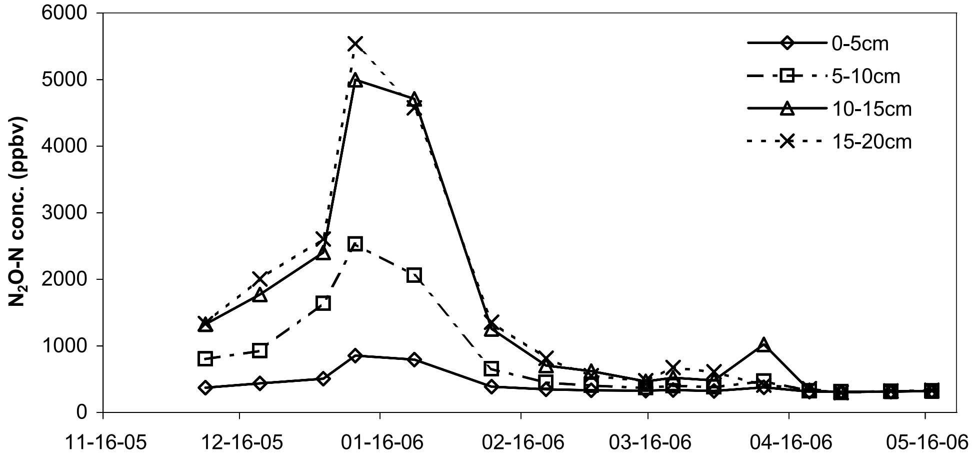


Figure 1. Temporal changes of soil gas nitrous oxide concentrations at different depths in a paddy soil during the drained crop growing season (n = 24; average CVs were 0.19, 0.36, 0.53, and 0.47 at 0–5 cm, 5–10 cm, 10–15 cm, and 15–20 cm).

missed the first twenty days of soil gas observations after wheat sowing, and caught up with the following observations starting 8 December 2005.

[14] Limited by the cost of making measurements, only one soil gas profile was taken for each treatment of the rice season. These samples were used to analyze the isotopic composition of N_2O three times during the first wheat-growing season, yielding a total of 96 soil gas samples. Profiles of soil ammonium, nitrate concentrations, and organic matter contents were simultaneously measured with N_2O isotope samples at depths of 0–5 cm, 5–10 cm, 10–15 cm, and 15–20 cm. Soil redox potential and temperature at these same four depths were recorded and converted to the standard H_2 electrode according to the corresponding soil temperature [*Faulkner et al.*, 1989]. These were monitored at the same plots as N_2O isotope samples with the same frequency as N_2O flux measurements.

[15] On the basis of the observations of the first wheat crop, we measured soil gas N_2O concentrations at the main production depth of 10–15 cm for the second wheat crop from eight soil profiles over the entire wheat growing season (for a total of eight observation dates). We also measured the soil gas N_2O isotopes at the 10–15 cm depth once during the flooded rice season in 2 July 2006. The supplementary isotopic compositions of soil nitrate, ammonium, and total N in the experimental field were also measured in triplicate at a depth of 0–15 cm on 1 June 2007.

2.4. Stable Isotope Analysis

[16] The ratios of the stable isotopes $^{15}N/^{14}N$ and $^{18}O/^{16}O$ in N_2O were determined by a MAT 253 isotope mass spectrometer using an online GC-IRMS system, consisting of a trace gas cryogenic preconcentration device (PreCon, Thermo Finnigan), gas chromatograph (GC, Thermo Finnigan) with PoraPLOT Q capillary column (0.32 mm × 25 m), and an isotope ratio mass spectrometer (Thermo-Finnigan MAT 253). The ISODAT 2.0 software suite was used for system control, data acquisition, and data evaluation. Isotopic data are reported as δ values, where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$. $R = {}^{15}N/^{14}N$ or $^{18}O/^{16}O$. Delta values are reported as deviations from $\delta^{15}N$ of atmospheric N_2 and $\delta^{18}O$ of standard mean ocean water (SMOW) ($\delta^{18}O_{SMOW}$). The conversion for the $\delta^{18}O_{atm}$ standard to $\delta^{18}O_{SMOW}$ standard is $\delta^{18}O_{atm} = -23 + \delta^{18}O_{SMOW}/1.0235$ [*Kim and Craig*, 1990]. The precision of the isotopic composition measurements in N_2O was better than 0.2‰ for ^{15}N and better than 0.5‰ for $\delta^{18}O$.

2.5. Presentation of Results

[17] Results are given as means with standard deviation and difference between depths and dates. They were analyzed according to Duncan's new multiple range test at $P < 0.05$ with SPSS 12.0 for Windows. Unless indicated otherwise, differences were only considered significant when $P < 0.05$. We define an emission peak as an emission flux significantly higher than the previous and following fluxes. Figures 1–5 are plotted as averages without standard deviation bars for clarity, and an average coefficient of variation (CV) across measurements for variables are provided in the captions.

3. Results

3.1. N_2O Concentrations Along Soil Profiles

[18] N_2O concentrations varied greatly over the wheat-growing season (Figure 1). The average N_2O concentrations peaked at 5.5 ml L^{-1} on 10 January 2006 and leveled off near the end of the season at an atmospheric concentration of 300 $\mu L\ L^{-1}$. The profile concentrations at each depth all peaked ($P < 0.05$) on 10 January, with an additional peak on 10 April at the 10–15 cm depth (Figure 1).

[19] N_2O concentrations are all significantly correlated between each soil layer during the whole observation period ($P < 0.05$). Within soil profile of 0–20 cm, higher N_2O concentrations were always observed at the lower layers of 10–15 cm/15–20 cm. This indicates that the lower profile is the main production zone throughout the wheat-growing season ($P < 0.05$) (Figure 1).

3.2. The $\delta^{15}N$ and $\delta^{18}O$ in N_2O Along Soil Profiles

[20] The $\delta^{15}N$-N_2O values increased from 0.7‰ at the 10–15 cm depth to 5.6‰ at the 0–5 cm depth on sampling

date of 6 March 2006. The δ^{15}N-N_2O values generally increased and the difference in δ^{15}N-N_2O values between soil depths decreased near the end of the wheat-growing season (Table 1). Soil gas at 10–15 cm depth produced the most depleted ^{15}N-N_2O during our measurement period, whereas the difference between sampling dates and soil depths was smaller for δ^{18}O-N_2O in soil profiles (Table 1).

Table 1. Natural Isotopic Abundance of N_2O and Concentrations of Soil Ammonium, Nitrate, and Organic Matter Content at Different Depths of a Paddy Soil During the Drained Crop Growing Season of the First Wheat Crop[a]

	$\delta^{15}N_{air}$ (‰)			$\delta^{18}O_{SMOW}$ (‰)			NH_4^+ (mg N kg^{-1})			NO_3^- (mg N kg^{-1})			Organic C (g C kg^{-1})	
Depth (cm)	6 March 2006	24 March 2006	18 April 2006	6 March 2006	24 March 2006	18 April 2006	6 March 2006	24 March 2006	18 April 2006	6 March 2006	24 March 2006	18 April 2006	6 March 2006	24 March 2006
0~5	5.6 ± 0.6 (a)	5.5 ± 0.9 (a)	5.7 ± 0.6 (a)	44.0 ± 2.4 (a)	41.8 ± 1.9 (a)	44.6 ± 4.7 (a)	45.4 ± 20.6 (a)	39.2 ± 14.7 (a)	3.3 ± 0.9 (a)	25.0 ± 7.3 (a)	35.5 ± 22.1 (a)	8.0 ± 2.7 (a)	11.6 ± 0.7 (a)	12.2 ± 0.5 (a)
5~10	3.1 ± 2.6 (ab)	4.5 ± 1.1 (ab)	5.6 ± 0.7 (a)	44.7 ± 5.0 (a)	42.2 ± 2.2 (a)	43.0 ± 4.7 (a)	9.0 ± 3.7 (b)	5.2 ± 1.4 (b)	1.9 ± 0.2 (b)	14.6 ± 6.3 (b)	12.8 ± 6.2 (b)	6.1 ± 2.7 (ab)	11.6 ± 0.7 (a)	12.3 ± 0.7 (a)
10~15	0.7 ± 5.1 (b)	2.6 ± 2.5 (c)	4.9 ± 1.3 (b)	45.6 ± 2.7 (a)	43.8 ± 2.8 (a)	41.6 ± 1.4 (a)	3.8 ± 1.2 (b)	2.5 ± 0.9 (b)	1.4 ± 0.2 (bc)	11.1 ± 4.9 (bc)	9.2 ± 2.9 (b)	4.5 ± 1.5 (bc)	10.5 ± 1.0 (b)	11.1 ± 1.1 (b)
15~20	1.6 ± 3.2 (b)	3.4 ± 1.6 (bc)	4.8 ± 0.6 (b)	46.5 ± 1.8 (a)	43.7 ± 2.9 (a)	42.1 ± 1.1 (a)	3.0 ± 0.8 (b)	1.2 ± 0.5 (b)	1.1 ± 0.3 (c)	8.4 ± 2.5 (c)	8.3 ± 1.8 (b)	3.8 ± 0.6 (c)	8.3 ± 1.4 (c)	9.0 ± 0.9 (c)

[a] Mean ± standard deviation for individual date, n = 8. Different letters within the same column indicate that the mean difference is significant at the $\alpha = 0.05$ level, by LSD test.

3.3. Soil Ammonium, Nitrate, Organic Matter, and Eh and Moisture Dynamics

[21] Concentrations of soil ammonium, nitrate, and organic matter along profiles were measured simultaneously with N_2O isotopes. Both soil ammonium N and nitrate N were concentrated at the top 0–5 cm layer compared to the lower three levels, between 5 cm and 20 cm ($P < 0.05$) (Table 1). The concentration difference is attributed to broadcasting. This difference was less pronounced with soil depth in the 18 April measurement, indicating that the wheat crop consumed most of the soil mineral N. Unlike soil mineral N, soil organic matter was quite stable during the observation period. Soil organic matter was higher at 0–10 cm than that at 10–15 cm, and the levels at 15–20 cm were the lowest in the profile ($P < 0.05$) (Table 1). These results all indicate that the upper soil layers are more conducive to soil microorganism growth and thus to N transformations.

[22] Soil redox potential (Eh), moisture, and surface N_2O flux were monitored throughout the entire observation period. The overall soil Eh results averaged more then 200 mV during the wheat-growing season (Figure 2a), indicating that the bulk of the soil was aerobic. Soil Eh values increased greatly when the field was changed from a flooded rice crop to a drained wheat crop. The 0–5 cm layer became positive earliest, followed by the 5–10 cm, 10–15 cm and 15–20 cm layers in order. There was large decrease in soil Eh on 10 April and 27 April due to precipitation (Figure 2c).

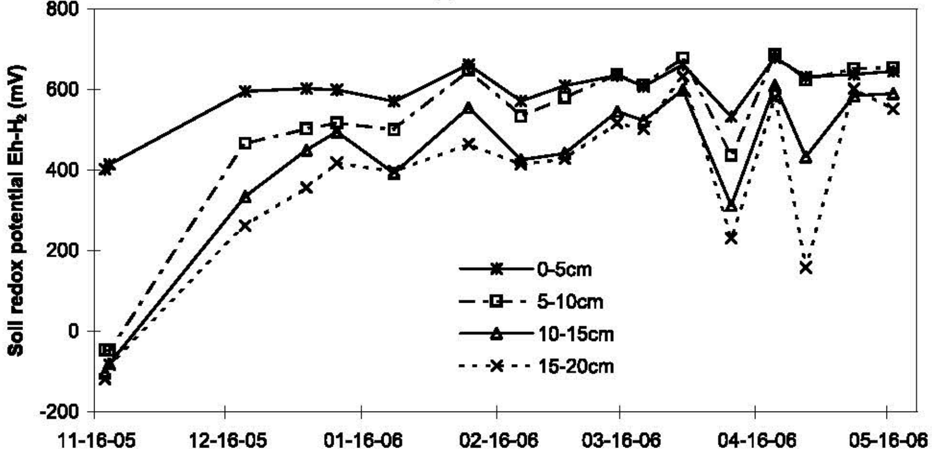
[23] The soil volumetric water in profile 0–20 cm fluctuated between approximately 0.28 $cm^3\ cm^{-3}$ and 0.34 $cm^3\ cm^{-3}$ during the main growth period (Figure 2b). This moderately moist soil condition throughout the season is conducive to nitrification.

3.4. Seasonal Dynamics of N_2O Fluxes and Related Soil Inorganic N

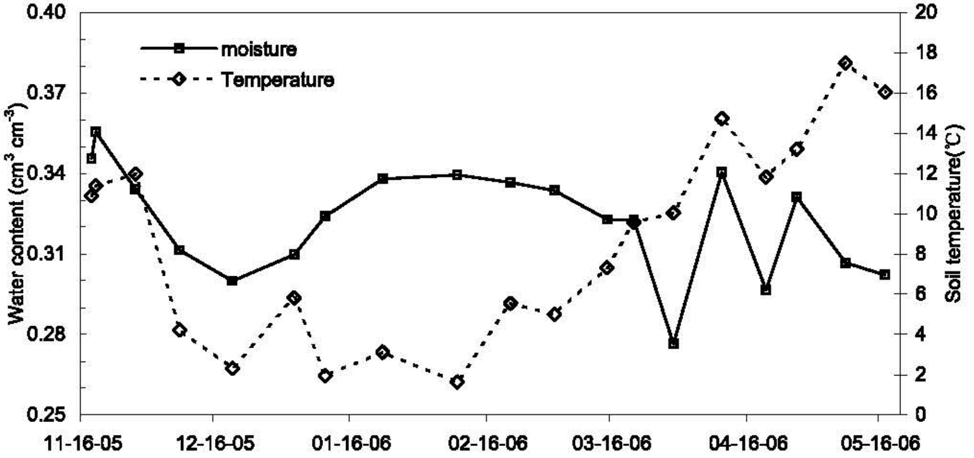
[24] The average surface N_2O fluxes fluctuated between -1.6 and 37.2 $\mu g\ N\ m^{-2}\ h^{-1}$ throughout the observation period. The average covariance of variations in surface flux was as high as 176%. Three emission peaks ($P < 0.05$) were detected in this study (Figure 3). The apparent relationship between the N_2O concentration gradient (Figure 1) and surface flux (Figure 3) is weak, and other factors may control surface N_2O emissions. Our measurements reveal that nitrous oxide emissions occur mainly in pulses, as opposed to continuously throughout the season.

[25] Changes in soil ammonium content reflect urea fertilizations. Soil ammonium concentrations reached the highest point when urea fertilizer was applied, and decreased thereafter (Figure 3). The increase of soil nitrate concentration after fertilization indicates strong nitrification activity in this soil, which is consistent with the observed aerobic soil Eh and moist conditions (Figure 2). The rapid

(a) Soil Eh



(b) Soil moisture-Temperature



(c) Daily Precipitation

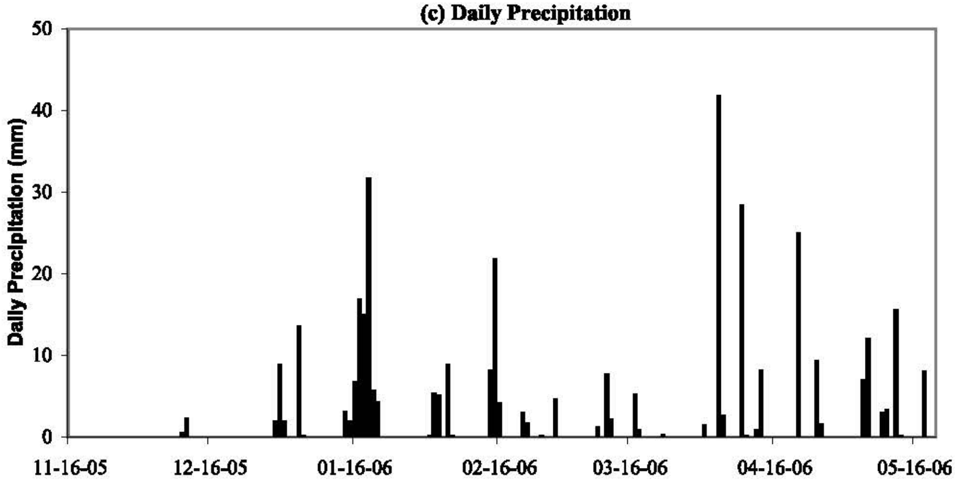


Figure 2. (a) Temporal changes of soil redox potential at different depths (n = 8), (b) soil volumetric water content and soil temperature (n = 24) in a paddy soil, and (c) daily precipitation from nearby weather station during the drained crop growing season (average CVs for Eh were 0.13, 0.12, 0.34, and 0.45 at 0–5 cm, 5–10 cm, 10–15 cm, and 15–20 cm).

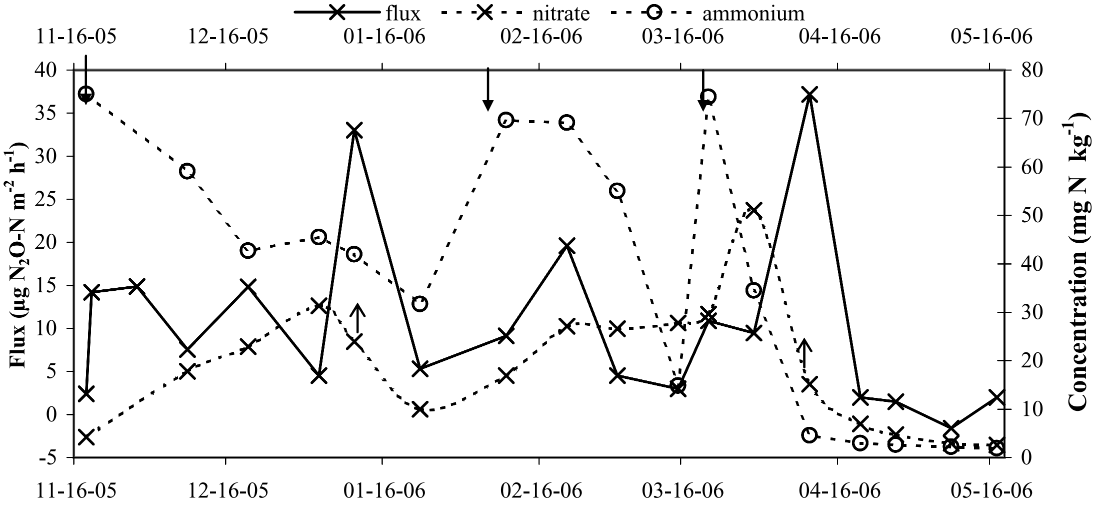


Figure 3. Temporal changes in nitrous oxide emissions, nitrate concentrations, and ammonium concentrations (n = 24) at the 0–15 cm depth of a paddy soil during the drained crop growing season. Average CVs for flux, nitrate, and ammonium were 1.76, 0.32, and 0.45, respectively. The solid arrow indicates the time of fertilizer application, and the open arrow indicates the concurrence of emission peak and nitrate decrease.

decrease in soil nitrate concentrations (Figure 3) that follows later may indicate strong denitrification activity, though neither nitrate loss through leaching nor crop uptake could be ruled out in this study.

3.5. Seasonal δ^{15}N-N_2O and δ^{18}O-N_2O Signatures in Rice-Based Ecosystems

[26] N_2O isotopic signatures were deduced from seasonal averages in the second wheat crop, which included eight sampling dates and eight soil profiles. The δ^{15}N and δ^{18}O values in N2O from soil gas at the 10–15 cm depth from the second wheat season averaged +0.90 ± 2.9 (n = 64) and +39.3 ± 3.1 (n = 64), respectively (Figure 4a). The δ^{15}N-N_2O values fluctuated between −6.3‰ and +5.9‰ throughout the entire wheat season. The temporal variation in δ^{15}N-N_2O values reached 12.2‰. The δ^{18}O-N_2O values fluctuated between 35.6‰ and 43.4‰, values smaller than the temporal difference in δ^{15}N-N_2O (Figure 4a).

[27] The objective of the second round of wheat crop measurements was to capture soil gas N_2O isotopic signatures covering the whole crop season, particularly for high soil gas N_2O concentrations, which were missed for the first wheat crop. During the second wheat season, N_2O concentrations were more variable over time (Figure 4b). The N_2O concentrations were low (at around 1.1 ml L^{-1}) initially, and increased rapidly to 5.9 ml L^{-1} within ten days of sowing. The concentrations remained elevated until the end of January 2007. One more soil gas concentration peak was observed on 5 March 2007, and the concentration leveled off near the end of this season. This high soil gas N_2O concentration was comparable to the first wheat crop (Figure 1). The δ^{15}N-N_2O values were lower for the second wheat crop than those for the first (Table 1).

[28] For comparison, we also measured the isotopic composition of soil gas at 10–15 cm during the flooded rice season in July 2006. The values of −0.57‰ for δ^{15}N-N_2O and +38.8‰ for δ^{18}O-N_2O were comparable to the averages from the wheat season (Figure 4a). To provide background information on the substrates, δ^{15}N values of soil ammonium, nitrate, and organic matter at the 0–15 cm depth from the experimental site were analyzed in triplicate in June 2007. Their values were −6.5‰, +11.8‰, and +6.3‰, respectively.

4. Discussion

[29] In the rice-based ecosystem, steep concentration gradients were observed throughout the entire sampling period, except at the end of the wheat-growing season (Figure 1). The observation that N_2O concentrations were much higher at the low depth of 10–20 cm ($P < 0.05$) (Figure 1), and that δ^{15}N-N_2O was more depleted at this depth as well (Table 1), suggest that this lower layer was the main N_2O production zone in the paddy field. Though the upper soil layer retained most of the soil ammonium, nitrate, and organic matter (Table 1), the main N_2O production occurred deeper in the soil profile (10–20 cm). The small amount of NO_3- in the lower soil layers is therefore sufficient for significant N_2O production, and N_2O production is not dependent on soil NO_3- concentration (Table 1 and Figure 1). Similarly, *Dendooven and Anderson* [1994] and *Ellis et al.* [1996] found that very low NO_3 soil concentrations can result in high N_2O production. *Müller et al.* [2004] also found that a small portion of the N applied to grassland soil in the summer was transported below the main rooting zone (>20 cm) and ultimately produced large amounts of N_2O at depths of 20–50 cm.

4.1. N_2O Production Processes: Denitrification Compared to Nitrification

[30] The various pathways in nitrification and denitrification produce N_2O with different isotopic signatures [*Yoshida*, 1988; *Yoshinari*, 1990; *Kim and Craig*, 1993]. These signatures help determine the mix of nitrification and denitrification occurring in the soil [*Pérez et al.*, 2006]. Nitrification often produces ^{15}N-depleted N_2O compared to

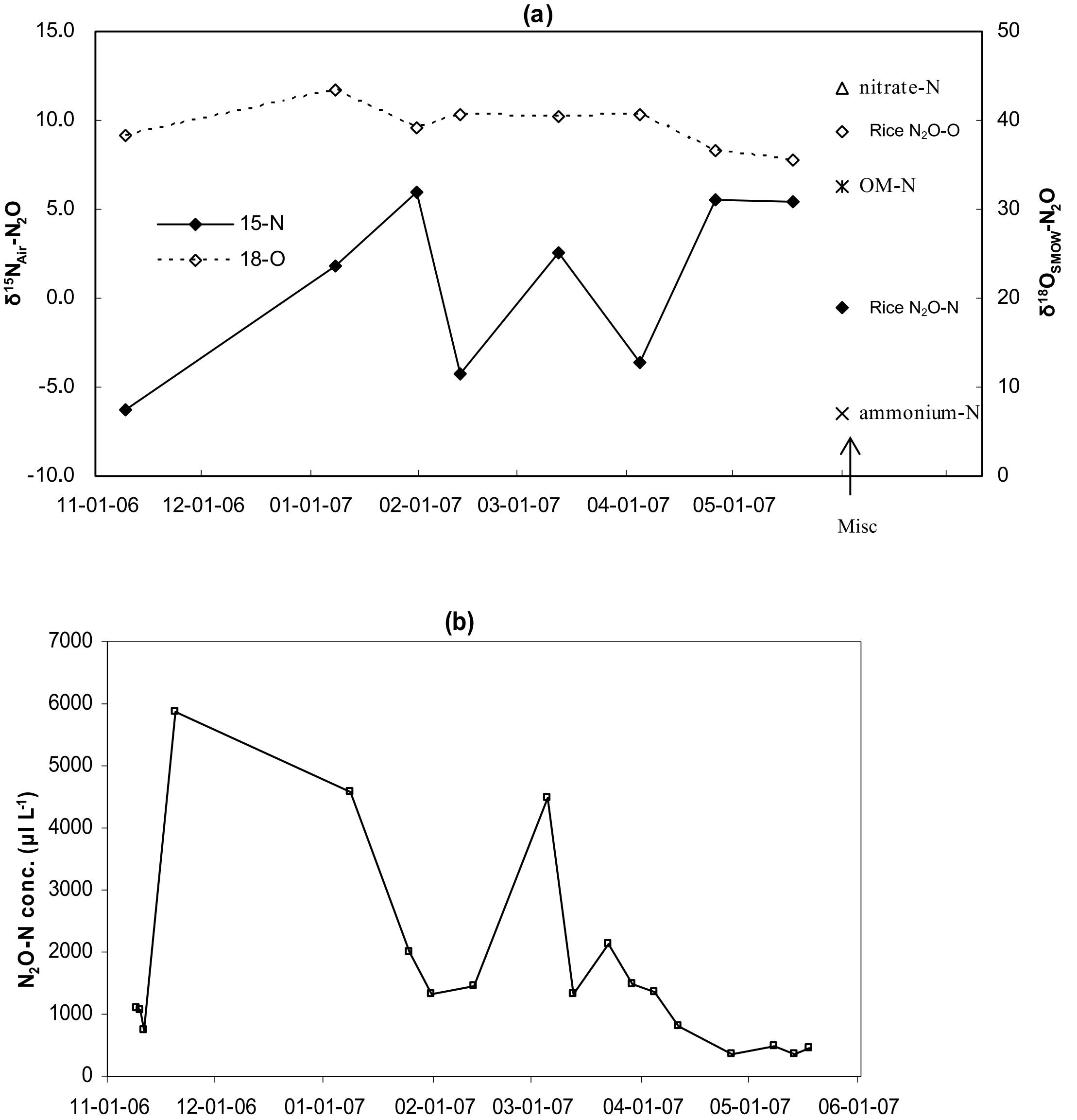


Figure 4. Temporal changes in (a) $\delta^{15}N_{air}-N_2O$ and $\delta^{18}O_{SMOW}-N_2O$ (n = 8); (b) N_2O concentrations (n = 8) in soil gas at the soil depth of 10–15 cm during the drained crop growing season of the second wheat crop (average CVs for $\delta^{15}N_{air}-N_2O$, $\delta^{18}O_{SMOW}-N_2O$, and N_2O concentrations were 0.57, 0.08, and 0.68, respectively). Soil gas $\delta^{15}N_{air}-N_2O$ and $\delta^{18}O_{SMOW}-N_2O$ (n = 8) at the 10–15 cm depth during the flooded rice-growing season of July 2006, $\delta^{15}N$ values (n = 3) of soil nitrate (+11.8 ± 1.0), ammonium (−6.5 ± 0.1), and soil organic matter (+6.3 ± 0.5) collected at 0–15 cm depth during the drained wheat season of June 2007 are also plotted in Figure 4a for supplementary information.

denitrification. The enrichment factors (difference in the ^{15}N between product and substrate) for the isotopic depletion of ^{15}N with nitrification ($NH_4^+ \rightarrow N_2O$) range from −45‰ to −66‰ [*Yoshida*, 1988]. N_2O produced via denitrification has two characteristic enrichment factors that reflect the role of N_2O as an intermediate in this process: −13‰ to −28‰ for the $NO_3^- \rightarrow N_2O$ step, and −13‰ to −27‰ for the $N_2O \rightarrow N_2$ step [*Yoshinari*, 1990; *Barford et al.*, 1999]. We can therefore reasonably expect that ^{15}N-N_2O derived from denitrification will range from −28‰ to +14‰, depending on the extent of N_2O reduction. Reduction of N_2O to N_2 preferentially removes the lighter isotope, which successively enriches the remaining N_2O in ^{15}N [*Barford et al.*, 1999; *Pérez et al.*, 2000; *Ostrom et al.*, 2000; *Vieten et al.*, 2007]. Consequently, nitrifier-derived ^{15}N-N_2O should be about 30‰ more negative than denitrifier-derived ^{15}N-N_2O if the ^{15}N values of soil nitrate and ammonium are equal.

[31] On the basis of this study's measurements of soil nitrate (+11.8‰) and ammonium (−6.5‰), we can expect ^{15}N-N_2O derived from nitrifiers to fall within −51.5‰ to −72.5‰ without the further step of $N_2O \rightarrow N_2$ reduction. Even if 99% of N_2O is reduced to N_2, the remaining ^{15}N-N_2O values should fall within −24.5‰ to −59.5‰. Similarly, ^{15}N-N_2O derived from denitrifiers should range from −1.2‰ to −17.2‰ without an additional reduction from $N_2O \rightarrow N_2$. If 99% of N_2O is reduced to N_2, then the

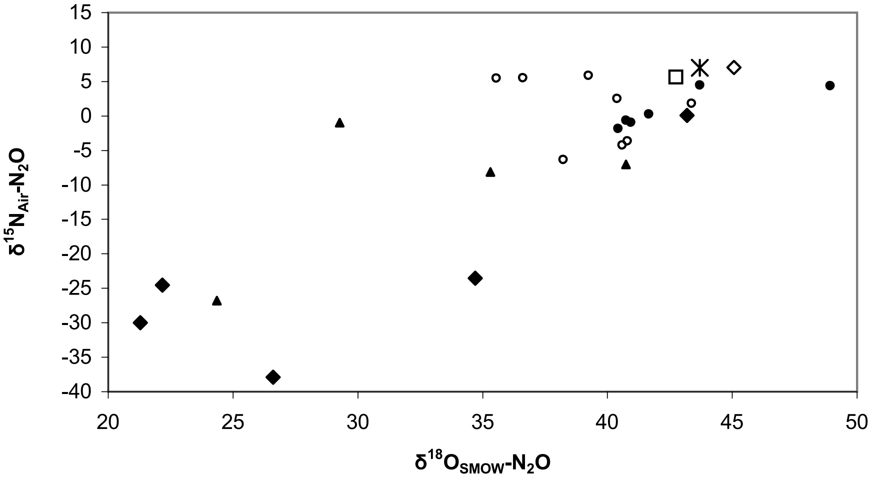


Figure 5. N_2O isotopic composition of the troposphere and N_2O sources. Average $\delta^{15}N$ and $\delta^{18}O$ values in N_2O from ambient air (n = 3, open diamond), soil-emitted air (n = 48, open square), and soil gases during the drained wheat season (n = 8) are here compared with previous studies to provide a global perspective. The six solid circles are from primary forest soil pits [*Pérez et al.*, 2000]; the five solid diamonds are results from fertilized wheat in Yaqui Valley [*Pérez et al.*, 2001], a papaya plantation in Costa Rica [*Pérez et al.*, 2000], a temperate lawn near San Diego, California [*Casciotti et al.*, 1997], and sugarcane fields on Maui island [*Kim and Craig*, 1993]. The solid triangles are from unfertilized tropical rain forest soils in Costa Rica and Brazil [*Pérez et al.*, 2000]. The average isotopic value for tropospheric N_2O is represented by a star.

remaining ^{15}N-N_2O values should be within −4.2‰ to +25.8‰. The ^{15}N-N_2O from our measurement (Table 1 and Figure 4) therefore falls within the enrichment factor reported for denitrification, not nitrification. However, ^{15}N measurements for soil nitrate and ammonium were taken on only one date and may not be representative of the entire season.

[32] The measurements of $\delta^{18}O$-N_2O (Table 1 and Figure 4) also support the conclusion deduced from $\delta^{15}N$-N_2O measurements in this study. Since nitrification involves oxidation, the $\delta^{18}O$ of emitted N_2O should be more depleted in ^{18}O than H_2O and O_2. For example, the $\delta^{18}O$ of ~22‰ in N_2O produced predominantly by nitrification in a wastewater treatment facility was significantly lower than the +43.7‰ value for atmospheric N_2O [*Yoshinari and Wahlen*, 1985]. Variation is expected in $\delta^{18}O$ values in N_2O from denitrification, though there should be correlation with the extent of removal of N_2O by reduction. The $\delta^{18}O$ value of N_2O derived from unfertilized soil was similar to that derived from the atmosphere, and the highest value of +93‰ was attributed to enrichment of $\delta^{18}O$ by denitrification [*Yoshinari and Wahlen*, 1985]. However, N_2O in the paddy soil was possibly derived from either denitrification alone or nitrification and denitrification combined. In both cases, the isotopic enrichment of N_2O must have occurred during the reduction of N_2O to N_2 [*Ostrom et al.*, 2000; *Menyailo and Hungate*, 2006; *Vieten et al.*, 2007]. Future work characterizing the ^{18}O isotopic composition of N_2O precursors will enable a better understanding of the $\delta^{18}O$-N_2O signature of these soils.

[33] Since the bulk soil in this study is aerobic (Figure 2a) and moderately moist (Figure 2b) during the drained wheat-growing season in this rice-based ecosystem, conditions were conducive to nitrification rather than denitrification. Elevated nitrification activity was also detected via a rapid NO_3^- concentration increase upon the application of urea (Figure 3). The rapid gross nitrification rates in the soil likely prevented nitrite (NO_2^-) accumulation [*Stark and Stephen*, 1997], a necessary precursor for N_2O formation through nitrification [*Wrage et al.*, 2001]. The N_2O production sites in lower soil layers coincided with a relatively lower soil Eh at these depths, which suggests that N_2O in rice paddy soil was probably produced by bacterial reduction processes operating in anaerobic niches [*Conrad*, 1996].

[34] This study reveals that nitrification makes a negligible contribution to the observed N_2O emissions from rice paddy soils over an annual period, regardless of whether the soil is flooded or drained (Figure 4a). Similar results were obtained for soil studies in a temperate grassland [*Müller et al.*, 2002, 2004] and an acid forest [*Wolf and Brumme*, 2002], both of which identified denitrification as the primary process generating N_2O.

4.2. Implications for the Global N_2O Budget

[35] We compare our isotopic measurements with published values from different soils and sites in Figure 5. On the basis of existing measurements, *Pérez et al.* [2001] attributed an overall $\delta^{15}N$-N_2O isotope average of −36.6 ± 9.2‰ (n = 21) to an agricultural soil source N_2O signature. However, this value is 37‰ more depleted compared to our paddy soil measurements ($\delta^{15}N$ = +0.90‰) (Figure 4a). Our results are still consistent with general observations that agricultural soil source N_2O is ^{15}N depleted relative to atmospheric N_2O [*Rahn and Wahlen*, 1997; *Pérez et al.*, 2000, 2001].

[36] The N_2O source from a rice-based ecosystem is much more enriched in $\delta^{15}N$ compared to other fertilized agricultural soil sources (Figure 5). Considering that rice

agriculture receives large amounts of synthetic fertilizer [*Zhu et al.*, 2005; *Xiong et al.*, 2008], agricultural source N_2O from rice versus other crops should have significantly different global isotopic signatures. Furthermore, the overall isotopic effect from agriculture is presumed to be underestimated since current isotopic signatures do not incorporate results from rice agriculture [*Rahn and Wahlen*, 2000; *Pérez et al.*, 2001].

[37] Published data on $\delta^{15}N-N_2O$ averages range between −37.9‰ and +4.5‰ (Figure 5), with the high variability attributed to processes occurring regardless of fertilization. Limiting data to fertilized soils, $\delta^{15}N-N_2O$ still varied from −37.9‰ [*Pérez et al.*, 2000] to +0.1‰ [*Kim and Craig*, 1993]. In part, the variability is due to soil moisture. *Kim and Craig* [1993] observed large spatial variability in soil $\delta^{15}N\text{-}N_2O$, with +0.1‰ from an abundant rainfall site and −23.5‰ from a dry soil site at a Maui island fertilizer-treated sugarcane field. Dramatic shifts in $\delta^{15}N\text{-}N_2O$ after fertilization have also been reported [*Pérez et al.*, 2001], ranging from a highly depleted value of −46.6‰ to an enriched value of +5‰. For unfertilized soil, $\delta^{15}N-N_2O$ results can also vary greatly; from −26.8‰ to −0.97‰ [*Pérez et al.*, 2000]. Our paddy soil measurements agree well with results from primary forest soil pits and unfertilized tropical rain forest soils in Brazil ($\delta^{15}N$ = −1.8‰ ~ +4.5‰) [*Pérez et al.*, 2000], and also with results from fertilized sugarcane fields on the wet site of Maui Island ($\delta^{15}N$ = +0.1‰) [*Kim and Craig*, 1993].

5. Conclusions

[38] Measurements of $\delta^{15}N$ and $\delta^{18}O$ in soil gas N_2O, in concert with profiles of N_2O concentration, surface N_2O flux, and soil mineral N, indicate that denitrification is the dominant process for N_2O production under the aerobic, wheat-cropping conditions found in rice-based ecosystems. Within the depth profile range of 0–20 cm, N_2O is primarily produced at the lower depths (10–15 cm), where both mineral N and organic matter are lower than in upper layers. The $\delta^{15}N$ and $\delta^{18}O$ signatures in soil gas N_2O from the rice-based ecosystem studied averaged +0.90‰ ($\delta^{15}N$) and +39.3‰ ($\delta^{18}O$) during the drained wheat-growing season, and −0.57‰ ($\delta^{15}N$) and +38.8‰ ($\delta^{18}O$) during the flooded rice-growing season. These values are significantly heavier in $\delta^{15}N$ and ^{18}O compared to other agricultural soil source N_2O. In summary, former estimations of agricultural soil source N_2O are underestimated since isotopic constraints from rice agriculture have not previously been considered.

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