Early season $N_2O$ emissions under variable water management in rice systems: source-partitioning emissions using isotopocule signatures along a depth profile

Elizabeth Verhoeven$^1$, Matti Barthel$^1$, Longfei Yu$^2$, Luisella Celi$^3$, Daniel Said-Pullicino$^3$, Steven Sleutel$^4$, Dominika Lewicka-Szczebak$^5$, Johan Six$^1$, Charlotte Decock$^1$

$^1$Department of Environmental Systems Science, ETH Zurich, 8092 Zurich, Switzerland  
$^2$Department of Air Pollution and Environmental Technology, EMPA, 8600 Dübendorf, Switzerland  
$^3$Department of Agricultural, Forest and Food Sciences, University of Turin, 10095 Grugliasco, Italy  
$^4$Department of Soil Management, Faculty of Bioscience and Engineering, 9000 Ghent University, Belgium  
$^5$Thünen Institute of Climate-Smart Agriculture, 38116 Braunschweig, Germany  
$^6$Department of Natural Resources Management and Environmental Sciences, California State University, 93407 San Luis Obispo, California

Correspondence to: Elizabeth Verhoeven (elizabeth.verhoeven@gmail.com)

Abstract. Soil moisture strongly affects the balance between nitrification, denitrification and $N_2O$ reduction and therefore the nitrogen (N) efficiency and N losses in agricultural systems. In rice systems, there is a need to improve alternative water management practices, which are designed to save water and reduce methane emissions, but may increase $N_2O$ and decrease nitrogen use efficiency. In a field experiment with three water management treatments, we measured $N_2O$ isotopocule signatures ($\delta^{15}N$, $\delta^{18}O$ and site preference, SP) of emitted and pore air $N_2O$ over the course of six weeks in the early rice growing season. Isotopocule measurements were coupled with simultaneous measurements of pore water $NO_3^-$, $NH_4^+$, dissolved organic carbon (DOC), water filled pore space (WFPS) and soil redox potential (Eh) at three soil depths. We then used the relationship between SP x $\delta^{18}O$-$N_2O$ and SP x $\delta^{15}N$-$N_2O$ in simple two endmember mixing models to evaluate the contribution of nitrification, denitrification, fungal denitrification to total $N_2O$ emissions and to estimate $N_2O$ reduction rates. $N_2O$ emissions were higher in a dry-seeded + alternate wetting and drying (DS-AWD) treatment relative to water-seeded + alternate wetting and drying (WS-AWD) and water-seeded + conventional flooding (WS-FLD) treatments. In the DS-AWD treatment the highest emissions were associated with a high contribution from denitrification and a decrease in $N_2O$ reduction; while in the WS treatments, the highest emissions occurred when contributions from denitrification/nitrifier-denitrification and nitrification/fungal denitrification were more equal. Modeled denitrification rates appeared to be tightly linked to nitrification and $NO_3^-$ availability in all treatments, thus water management affected the rate of denitrification and $N_2O$ reduction by controlling the substrate availability for each process ($NO_3^-$ and $N_2O$), likely through changes in mineralization and nitrification.

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rates. Our model estimates of mean N$_2$O reduction rates match well those observed in $^{15}$N fertilizer labeling studies in rice systems and show promise for the use of dual isotopocule mixing models to estimate N$_2$ losses.

1 Introduction

Atmospheric nitrous oxide (N$_2$O) concentrations continue to rise, and with a global warming potential 298 times that of CO$_2$, N$_2$O is a significant contributor to global warming (IPCC, 2007; Ravishankara et al., 2009). Agriculture is estimated to be responsible for roughly 60% of anthropogenic N$_2$O emissions (Smith et al., 2008). Considering this, the quantification of field scale N$_2$O emissions has been the focus of many studies in the last decades and much progress has been made on identifying agricultural management practices, soil and climate variables that influence emissions (Mosier et al., 1998; Ventera et al., 2012; Verhoeven et al., 2017). However, it remains difficult to quantitatively determine the biological sources of emitted N$_2$O in the field, and knowledge gaps remain in our understanding of how N$_2$O production and reduction processes change with both time and depth. More specific knowledge of process dynamics is therefore needed to inform and improve biogeochemical models.

N$_2$O is predominately produced 1) as a byproduct during nitrification, where NH$_4^+$ is oxidized to NO$_3^-$ via hydroxylamine (NH$_3$OH); this step of nitrification is sometimes referred to as hydroxylamine oxidation (Schreiber et al., 2012; Hu et al., 2015) or 2) as an intermediate in the denitrification pathway during which NO$_3^-$ is reduced to N$_2$ (Firestone et al., 1989) or 3) during nitrifier-denitrification by specific ammonia oxidizing bacteria that oxidize NH$_4^+$ to NH$_2$OH and then to NO$_2^-$, with a small fraction of NO$_2^-$ then being reduced to NO and N$_2$O (Wrage et al., 2001; Kool et al., 2010; Kool et al., 2011). N$_2$O may also be produced from additional biotic and abiotic processes, such as fungal denitrification, coupled nitrification-denitrification, dissimilatory nitrate reduction to ammonium, chemodenitrification or hydroxylamine decomposition (Butterbach-Bahl et al., 2013; Heil et al., 2015; Zhu-Barker et al., 2015). Due to the prevalence of anaerobic conditions and the use of NH$_4^+$ based fertilizers fungal denitrification and coupled nitrification-denitrification, respectively, are likely to increase in flooded rice systems. N$_2$O is consumed during the final step of denitrification, where N$_2$O is reduced to N$_2$ by the N$_2$O reductase pathway. This can occur sequentially within denitrifying organisms, or N$_2$O produced elsewhere from other processes or incomplete denitrification can be later reduced by denitrifiers. The final and dominant product of denitrification is N$_2$. While, N$_2$ emissions are not of concern for global warming, the quantification of gross denitrification rates is of environmental concern because the loss of N via this process may represent a loss of N from system and indicate reduced fertilizer N efficiency. Gross denitrification rates are difficult to measure in situ without the use of isotope tracers due to the high atmospheric background of N$_2$, thus denitrification and N$_2$ emissions remain a relatively unconstrained aspect of N budgets.

Studying N cycling in rice systems offers a unique opportunity to study processes of N$_2$O production and reduction. Firstly, the complex hydrology, and variable soil moisture conditions between soil layers and within the time course of a growing...
season, may induce a patchwork of conditions favorable for nitrification versus denitrification versus N$_2$O reduction. For example, it is not clear if low N$_2$O emissions under more moist conditions are the result of lower N$_2$O production due to substrate limitation (i.e. low nitrification rates and hence low NO$_3^-$) or rather increased N$_2$O reduction. To date, few studies have looked at N$_2$O processes at depth and it is not known how moisture and nutrient stratification affect the balance between N$_2$O production and consumption processes and ultimately surface emissions. Analysis of soil N$_2$O concentrations along a profile should help answer this. Secondly, there is a strong need to develop alternative water management practices with shortened paddy flooding period, in order to save water and mitigate methane (CH$_4$) emissions. However, such systems can cause an increase in N$_2$O emission that may partially offset the decrease in CH$_4$ emission (Devkota et al., 2013; Xu et al., 2015; Miniotti et al., 2016). Hence, water management practices should be improved based on a better understanding of the spatiotemporal origin of N$_2$O emissions and inorganic N precursors, nitrate and ammonium. Thirdly, rice cropping systems typically suffer from a lower nitrogen use efficiency (NUE) than other major cereal crops, often attributed to high gaseous NH$_3$ and N$_2$ losses (Dedatta et al., 1991; Cassman et al., 1998; Aulakh et al., 2001; Dong et al., 2012). In improving the NUE, a better estimate of N$_2$O reduction to N$_2$ is needed to design strategies that reduce N$_2$ losses without increasing N$_2$O emission.

The measurement of N$_2$O isotope signatures at natural abundance is a tool to differentiate between in situ N$_2$O source processes and N$_2$O reduction (Baggs, 2008; Ostrom and Ostrom, 2011; Toyoda et al., 2011; Wolf et al., 2015), i.e. N$_2$O source-partitioning. The evolution of analytical techniques now allows us to measure not only the bulk δ$^{15}$N-N$_2$O, but also the intermolecular distribution of the δ$^{15}$N within N$_2$O, called site-preference (SP) and the δ$^{15}$N of N$_2$O precursors, nitrate (NO$_3^-$) and ammonium (NH$_4^+$). The δ$^{18}$O of N$_2$O and its precursors may also be used to constrain processes (Kool et al., 2009; Lewicka-Szczebak et al., 2016; Lewicka-Szczebak et al., 2017). Analytical methods of interpretation remain, however, only semi-quantitative due to uncertainty surrounding net isotope effects (ε) for individual processes, overlap in the δ signatures between processes, and/or multiple N and O sources for which determination of δ$^{15}$N and δ$^{18}$O remains expensive and time consuming. Theoretically, the O in N$_2$O derives from O$_2$ during nitrification and from NO$_3^-$ during denitrification or a combination during nitrifier-denitrification (Kool et al., 2007; Kool et al., 2010; Snider et al., 2012, 2013; Lewicka-Szczebak et al., 2016). However, in the case of nitrifier-denitrification and denitrification, intermediates in the reduction pathway (NO$_2^-$ and NO) can extensively exchange O atoms with H$_2$O (Kool et al., 2007). Such exchange lowers the measured δ$^{18}$O-N$_2$O values because the influence of relatively depleted δ$^{18}$O from H$_2$O, potentially leading to an underestimation of denitrification and N$_2$O reduction (Snider et al., 2013; Lewicka-Szczebak et al., 2016). Indeed, it has been shown that the ε$^{18}$O for denitrification should be calculated relative to H$_2$O not NO$_3^-$, as almost 100% O exchange occurs (Lewicka-Szczebak et al., 2014; Lewicka-Szczebak et al., 2016). The use of δ$^{15}$N values is theoretically more straightforward and there is also a much richer body of literature on ε$^{15}$N for various processes, which was recently compiled and reviewed by Denk et al. (2017). The authors report a mean isotope effect for $^{15}$N during NH$_4^+$ oxidation to N$_2$O of -56.6 ± 7.3% and of -42.9 ± 6.3% for NO$_3^-$ reduction to N$_2$O. Additionally, accurate measurement of the δ$^{15}$N of NH$_4^+$ and NO$_3^-$ at sufficient temporal resolution remains time consuming. In comparison, the SP is thought to be independent of the initial substrate δ$^{15}$N values and shows distinct
values for two clusters of N$_2$O production, namely 32.8 ± 4.0‰ for nitrification/fungal denitrification/abiotic N$_2$O production and -1.6 ± 3.8‰ for denitrification/nitrifier-denitrification (Decock and Six, 2013a; Denk et al., 2017).

All three δ values are affected by N$_2$O reduction to N$_2$, which serves to enrich in heavy isotopes ($^{15}$N and $^{18}$O) the pool of remaining N$_2$O that is measured (Decock and Six, 2013a; Zou et al., 2014). If the δ value of N$_2$O$_{inal}$ (prior to reduction) can be reasonably estimated from graphical and mixing model approaches, then the subsequent enrichment of N$_2$O can be used to estimate N$_2$O reduction rates and thereby total denitrification rates. This is important because N$_2$O reduction is a crucial but exceptionally poorly constrained process within the N cycle (Lewicka-Szczebak et al., 2017). Fractionation during N$_2$O reduction may follow dynamics of open or closed systems (Mariotti et al., 1981; Fry, 2007). In open systems a continuous supply of fresh (and non-enriched) N$_2$O is assumed to enter the system, while in closed systems a given pool of N$_2$O is progressively used up. Closed system dynamics result in a greater enrichment of the residual N$_2$O pool and lower associated N$_2$O reduction rates. In reality in situ processes likely exhibit aspects of both systems heterogeneously in time and space (Decock and Six, 2013b).

Our goal was to collect a high resolution in situ N$_2$O isotopocules data set that could be used to a) determine the stratification of N$_2$O production and reduction processes in relation to water management, b) semi-quantitatively assess N$_2$O and N$_2$ losses among rice water management treatments and c) push forward current natural abundance N$_2$O isotope source-partitioning methods and interpretation at the field scale. We compared three rice water management practices: direct dry seeding followed by alternate wetting and drying (DS-AWD), wet seeding followed by alternate wetting and drying (WS-AWD) and wet seeding followed by conventional flooding (WS-FLD). Isotope data was determined at three depths, simultaneously with soil environmental and nutrient data and soil N$_2$O and dissolved N$_2$O concentrations. We hypothesized that N$_2$O emissions would be highest in the AWD treatments due to greater contributions from nitrification and less N$_2$O reduction, following the order: DS-AWD > WS-AWD > WS-FLD. We also hypothesized that N$_2$ emissions are controlled by the availability of NO$_3^-$ coming from nitrification and high soil moisture. We considered that NO$_3^-$ would be higher under WS-AWD but soil moisture would be higher under WS-FLD; therefore we predicted N$_2$ emissions to follow in the order: WS-AWD > WS-FLD > DS-AWD. Lastly, we hypothesized that longer periods of lowered soil moisture in the DS-AWD and WS-AWD treatments would result in greater production of N$_2$O at depth and this higher production would increase surface emissions.

2 Materials and Methods

2.1 Field experiment

A field experiment consisting of three water management regimes was conducted at the Italian Rice Research Center (Ente Nazionale Risi), Pavia, Italy (45°14’48”N, 8°41’52”E). Experimental work focused only on the early growing season, lasting
from the 13\textsuperscript{th} of May, 2016 until June 30\textsuperscript{th}, 2016. It is in this period that the highest N\textsubscript{2}O losses and N cycling dynamics had been previously observed and the largest differences among water management practices occurred. The experimental platform has been extensively described in previous publications (Miniotti \textit{et al.}, 2016; Peyron \textit{et al.}, 2016; Said-Pullicino \textit{et al.}, 2016; Verhoeven \textit{et al.}, 2018). The soil at the site has been classified as coarse silty, mixed, mesic Fluvaquentic Epiaquept (USDA-NRCS, 2010). The mean soil texture in the upper 30 cm of the experimental plots was 26\% sand, 62\% silt, and 11\% clay with a mean bulk density of 1.29 g cm\textsuperscript{3}. The mean total organic C and total N were 1.07 and 0.11\% and pH 5.9 (1:2.5 H\textsubscript{2}O) and 5.2 (1:2.5 0.01M CaCl\textsubscript{2}), respectively. Annual and growing season mean temperatures in 2016 were 10°C and 23°C, respectively (Fig. S1). Annual and growing season cumulative precipitation was 618 and 258 mm, respectively. Data for both values were retrieved from a regional weather station operated by the Agenzia Regionale per la Protezione dell’Ambiente-Lombardia, located approximately 200 m from the field site (ARPA).

Water management in the two WS treatments was identical during the first three weeks of the growing season (Table 1). Following regional practices for water seeding, paddies were flooded for six days at the time of seeding, but then drained for \sim 2 weeks to promote germination. During this period of ‘drainage’ paddies were not dry but maintained near saturation by flush irrigation as necessary (May 31\textsuperscript{st} and June 6\textsuperscript{th}). Flush irrigation is a practice in which the water inlet channels are opened for a few hours and then the outlet channels are opened a few hours later resulting in temporary soil saturation or even 1-2 cm ponding for 2-4 hours. On June 10\textsuperscript{th}, approximately three weeks after seeding, treatment differentiation between the WS-FLD and WS-AWD began. At this time the WS-FLD was flooded, while the WS-AWD was only flush irrigated. On June 16\textsuperscript{th}, the WS-FLD was allowed to drain slowly in order to facilitate fertilizer application on June 21\textsuperscript{st}. Following fertilizer application, the WS-FLD treatment was re-flooded and both AWD treatments were flush irrigated on June 22\textsuperscript{nd}. In the DS-AWD treatment no flooding or irrigation water was applied prior to June 22\textsuperscript{nd}. Soil moisture depended on rainfall, which was 75 mm during the four weeks following seeding.

In all treatments, crop residues were incorporated in the spring, before the cropping season. All paddies were harrowed and leveled approximately one month prior to seeding in mid-April, 2016. All treatments were pre-fertilized with phosphorus and potassium on May 13\textsuperscript{th} (14 and 28 kg ha\textsuperscript{-1}, respectively) and with urea on May 16\textsuperscript{th} (40 and 60 kg ha\textsuperscript{-1} for the DS and WS treatments, respectively). The DS-AWD treatment was seeded on May 17\textsuperscript{th}, 2016. The WS-FLD and WS-AWD treatments were seeded on May 20\textsuperscript{th}. All treatments were fertilized with urea on June 21\textsuperscript{st} (70 and 60 kg ha\textsuperscript{-1} for the DS and WS treatments, respectively). All treatments were harvested on September 15\textsuperscript{th}.

Each treatment consisted of two paddies, 20 x 80 m, with two plots in each paddy, n=4 (Fig. S2). The experimental design was identical to that of Verhoeven \textit{et al.} (2018), with the addition of the DS-AWD treatment and some adjustment to plot placement in order to accommodate data logging devices and field equipment. Each paddy was approximately 2 m apart and hydrologically separated by a levee of 50 cm above the soil surface, flanked by an irrigation canal on either side. Sampling for N\textsubscript{2}O surface fluxes, pore water parameters (NO\textsubscript{3}-, NH\textsubscript{4}+, DOC, dissolved N\textsubscript{2}O) and pore air N\textsubscript{2}O occurred on 15-17 dates,
from the 20th of May to the 30th of June, 2016 (Table S1). Sampling dates were on average three days apart with a greater frequency before and after N application on the 21st of June. Sub-samples of pore water from 10 to 12 dates were analyzed for δ15N-NO3, δ18O-NO3 and δ15N-NH4.

2.2 Soil environment: temperature, redox potential, and moisture

Soil moisture was measured using PR2 capacitance probes (Delta T Devices, UK) at 5, 15, 25, 45 and 85 cm. Water filled pore space (WFPS) was calculated using bulk density measurements at 5, 12.5 and 25 cm collected at the beginning of the season using a Giddings manual soil auger. Soil temperature was measured in only one plot per paddy (n=2) at three depths (5, 12.5 and 25 cm). Measurements were made manually at the time of surface flux gas measurements. Soil redox potential (Eh) was measured continuously in each plot using sturdy tip probes outfitted with 5 Pt-electrodes that were permanently connected to a 48-channel Hypnos-III data logger (MVH Consult, The Netherlands) with two Ag/AgCl-reference probes. Soil Eh was measured every hour at six depths; 5, 12.5, 20, 30, 50 and 80 cm. We took the average of the 20 and 30 cm readings to derive a 25 cm reading in order to correlate to other measurements.

2.3 N2O measurements: surface emissions, pore air, and dissolved gas

All N2O concentration measurements were measured by gas chromatography on a Scion 456-GC (Bruker, Germany) equipped with an electron capture detector (ECD). The error of the GC was determined to be ±0.012 at 0.3 ppm and ±0.024 ppm at 1.0 ppm. N2O surface emissions (N2O_remitted) were measured by the non-steady state closed chamber technique (Hutchinson and Mosier, 1981). The chamber design and deployment was identical to that of Verhoeven et al. (2018). Gas samples were taken at 0, 10, 20 and 30 min in each chamber and injected into pre-evacuated exetainers (Labco, UK). At time 0 and 30 min an additional ~170 ml of sample was taken and injected into gas crimp neck vials sealed with Butyl injection stoppers (IVA Analysentechnik, Germany) to be used for isotope analysis. When the accumulation of gas over the course of measurement was less than the GC error associated with the highest concentration of the four measurements, the flux was set to zero. Fluxes above the detection limit were calculated by linear or non-linear regression following the method outlined by Verhoeven and Six (2014). Soil N2O (N2O_soil) was sampled using passive diffusion probes installed at 5, 12.5 and 25 cm. The probe design and sampling strategy has been previously described in Verhoeven et al. (2018). In brief, the samples were collected in He flushed and pre-evacuated 100 ml glass crimp neck vials (actual volume 110 ml, IVA Analysentechnik, Germany) and after sampling topped with high purity He gas to prevent leakage into under-pressurized vials. The final N2O concentration was determined by gas chromatography, as described above, on a subsample, while the remainder of the sample was retained for isotope analysis. The final N2O concentration was calculated by accounting for sample dilution based on the pressure after evacuation, after sampling and after topping with He gas. Samples for dissolved N2O (N2O_dissolved) were collected by injecting a 5 ml subsample of pore water, collected as described in section 2.4, into N2 flushed and filled exetainers that also contained 50µl of 50% ZnCl to stop microbial activity. Samples were stored at 4°C until the end of the experimental campaign and transported back to the lab for analysis, therefore there was adequate time for the equilibration between the headspace and
aqueous phases. The molar concentration of N₂O was calculated by applying the solubility constant of N₂O at the time of analysis (i.e. lab temperature) to Henry’s law (Wilhelm et al., 1977; Weiss and Price, 1980; Lide, 2004), taking into account the vial volume and headspace.

2.4 Pore water measurements

Two MacroRhizon pore water samplers (Rhizosphere Research Products, The Netherlands) were installed at each depth (5, 12.5 and 25 cm) in every plot. Pore water was then collected in two polypropylene 60 ml syringes at each depth and later pooled together at sample processing. The syringes were attached to the MacroRhizon sample tubes with two-way leur lock valves and propped open using a wedge, which served to create a low vacuum; the syringes were left to collect water for 2-4 h. Samples were stored at 4°C and processed within 36 h. During pore water processing ~ 15 ml of solution was allocated for analysis of NO₃ and NH₄⁺ and δ¹⁵N, δ¹⁸O-NO₃, ~ 15 ml for δ¹⁵N-NH₄⁺, 5 ml for dissolved N₂O, 3-5 ml for dissolved Fe²⁺ and Mn²⁺ and 5 ml for DOC/TDN analysis. All samples, aside from those for dissolved N₂O, were frozen at -5°C until analysis. NO₃ and NH₄⁺ were determined by spectrophotometry following the procedure of (Doane and Horwáth, 2003). DOC and TDN were determined by first acidifying the water sample to pH <2 by addition of concentrated HCl and then analysis on a multi N/C 2100S:TOC/TN Analyzer (Analytik Jena, Germany).

2.5 Determination of δ¹⁵N, δ¹⁸O and isotopomer signatures in N₂Oemitted and N₂Osoil

Surface and pore air gas samples were taken in 100 ml glass crimp neck vials (actual volume 110 ml, IVA Analysentechnik, Germany) as described in section 2.3. Pore air gas samples were preconditioned with 1ml of 1M NaOH solution prior to analysis due to very high CO₂ concentrations in many samples (> 5000 ppm). The intramolecular site-specific isotopic composition of the N₂O molecule was measured using a gas preparation unit (Trace Gas, Elementar, UK) coupled to an isotope ratio mass spectrometer (IRMS; IsoPrime100, Elementar, UK). The gas preparation unit was modified with an additional chemical trap (½” diameter stainless steel), located immediately downstream from the autosampler. This pre-trap was filled with NaOH, Mg(ClO₄)₂, and activated carbon in the direction of flow and is designed to further scrub CO₂, H₂O, CO and VOCs which otherwise would cause mass interference during measurement. Before final injection into the IRMS the purified gas sample is directed through a Nafion drier and subsequently separated in a gas chromatograph column (5Å molecular sieve).

The IRMS consists of five Faraday cups with m/z of 30, 31, 44, 45, 46, measuring δ¹⁵N and δ¹⁸O of N₂O and δ¹⁵N from the NO⁺ fragments dissociated from N₂O during ionization in the source. The ¹⁵N/¹⁴N ratio of the NO molecule is used to calculate the α (central) position of the initial N₂O, thus allowing measurement of the site-specific isotopic composition of N₂O (SP). Site preference is defined as δ¹⁵N_{SP} = δ¹⁵N_{α} – δ¹⁵N_{β} with α denoting the ¹⁵N/¹⁴N ratio of the central N atom and β the ¹⁵N/¹⁴N ratio of the terminal N atom of the linear NNO molecule. δ¹⁵N_{β} is indirectly obtained from rearrangement of:

\[ δ^{15}N_{\text{bulk}} = \frac{δ^{15}N_{α} + δ^{15}N_{β}}{2} \]
which represents the average $^{15}$N content of the N$_2$O molecule.

For IRMS calibration three sets of two working standards (~ 3 ppm N$_2$O mixed in synthetic air) with different isotopic composition ($\delta^{15}$N$^\alpha$ = 0.954 ± 0.123 ‰ and 34.446 ± 0.179 ‰; $\delta^{15}$N$^\beta$ = 2.574 ± 0.086 ‰ and 35.98 ± 0.221 ‰; $\delta^{18}$O = 39.741 ± 0.051 ‰ and 38.527 ± 0.107 ‰) were used. These standards have been analyzed at EMPA using TREX-QCLAS versus standards with assigned δ-values by Tokyo Institute of Technology (Mohn et al., 2014). These working standards were run in triplicate, evenly spaced throughout a run. Sample peak ratios are initially reported against a N$_2$O reference gas peak (100% N$_2$O, Carbagas, Switzerland) and are subsequently corrected for drift and span using the working standards. Further correction procedures, such as $^{17}$O mass overlap and scrambling, as reported elsewhere, were not applied as the data was inherently corrected by regression between true and measured values of the triplicate working standards. Long-term measurement quality was ensured using a control standard at low N$_2$O concentration (~ 0.4 ppm) treated as a sample. Instrument linearity and stability was frequently checked by injection of 10 reference gas pulses of either varying or identical height respectively, with accepted levels of <0.03%/nA. Since instrument linearity could only be achieved for either N$_2$O or NO, the instrument had been tuned for the former and $\delta^{15}$N$^\alpha$ subsequently corrected using sample peak height assuming a non-linearity of 0.1 ‰ nA$^{-1}$. Such linearity complications have been previously reported using Elementar (Ostrom et al., 2007) and ThermoFinnigan IRMS (Röckmann et al., 2003). Tropospheric air was regularly measured (n=42) and used as a confirmation of correction procedures, yielding consistent and reliable results: $\delta^{15}$N$^{SP}$ = 18.77 ± 1.08 ‰; $\delta^{15}$N$^{bulk}$ = 5.96 ± 0.35 ‰; $\delta^{15}$N$^\alpha$ = 15.34 ± 0.70 ‰, $\delta^{15}$N$^\beta$ = -3.43 ± 0.60 ‰; $\delta^{18}$O = 43.67 ± 0.41 ‰. All $^{15}$N/$^{14}$N sample ratios are reported relatively to the international isotope ratio scale AIR-N2 while $^{18}$O/$^{16}$O are reported versus Vienna Standard Mean Ocean Water (V-SMOW). Relative differences are given using the delta notation (δ) in units of ‰:

$$\delta^ZX \ [\%o] = \frac{R_{sample}}{R_{reference}} - 1$$

(1)

where $R$ is referring to the molar ratio of $^{15}$N/$^{14}$N or $^{18}$O/$^{16}$O and $^ZX$ to the abundance of the heavy stable isotope Z of element X.

### 2.6 Determination of $\delta^{15}$N-NH$_4^+$, $\delta^{18}$O-NO$_3^-$ and $\delta^{15}$N-NH$_4^+$

Pore water NO$_3^-$ samples were analyzed for $\delta^{15}$N and $\delta^{18}$O at the University of California, Davis, Stable Isotope Facility (http://stableisotopenfacility.ucdavis.edu/), using the denitrifier method developed by (Sigman et al., 2001; Casciotti et al., 2002; McIlvin and Casciotti, 2011). $\delta^{15}$N-NH$_4^+$ in pore water was determined by micro-diffusion onto acidified disks followed by persulfate digestion (Stephan and Kavanagh, 2009; Lachouani et al., 2010) and lastly by the denitrifier method. For $\delta^{15}$N-NH$_4^+$, all steps and analyses were done in-house, including the denitrifier method. Briefly, samples were run in sets of 40 with 24 samples and a combination of 16 standards and blanks. Each run contained at least two $\delta^{15}$N-NH$_4^+$ isotope standards (IAEA N2 = 20.3‰; IAEA N1 = 0.4‰; USGS 25 = -30.4‰) at two or three concentrations in duplicate or triplicate in addition to...
two blanks and two working standards. NH₄⁺ isotope standards were diffused, digested and run through the denitrifier method in parallel with samples and therefore an overall correction and concentration offset was derived and applied for each batch. The denitrifier method was executed using the updated protocol described by McIlvin and Casciotti (2011) using Pseudomonas aureofaciens (ATCC 13985). An IAEA KNO₃ standard (δ¹⁵N = 4.7‰) was included at the denitrifier method step to ensure accurate conversion of NO₃⁻ to N₂O. A propagated error across all steps of δ¹⁵N-NH₄⁺ quantification was calculated from the working standards included in each batch (n=18). We excluded three values that were well outside the expected range; our overall precision was 1.9‰. The largest sources of error were incomplete diffusion or persulfate digestion. For δ¹⁵N-NO₃⁻ and δ¹⁸O-NO₃⁻ analyzed at SIF, UC-Davis, the limit of quantification was 2.0 μM NO₃⁻ or 0.125 mg L⁻¹ NO₃⁻, with a precision of 0.4‰ and 0.5‰ for δ¹⁵N and δ¹⁸O, respectively.

The net isotope effect (ε) of NO₃⁻ reduction to N₂O and for NH₄⁺ oxidation to N₂O and were calculated using equation 2 and 3, respectively.

\[ \varepsilon^{15}N_{N_2O-NO_3} = \delta^{15}N_{N_2O} - \delta^{15}N_{NO_3} \] (2)

\[ \varepsilon^{15}N_{N_2O-NH_4} = \delta^{15}N_{N_2O} - \delta^{15}N_{NH_4} \] (3)

2.7 Determination of N₂O source contribution and N₂O reduction

2.7.1 Two endmember mixing models using SP and δ¹⁸O signatures: closed and open systems

We tested two mixing models where N₂O reduction was modeled under ‘open’ and ‘closed’ system dynamics following the theory outlined originally by Fry (2007) and Mariotti et al. (1981), respectively. The two modelling methods are henceforth referred to as ‘open’ and ‘closed’. Additionally, we modeled two possible scenarios, as described by Lewicka-Szczebak et al., (2017); scenario 1 (sc1), where N₂O is produced and reduced by denitrifiers before mixing with N₂O derived from nitrification or scenario two (sc2) where N₂O is produced from both processes, mixed, and then reduced. In both models, N₂O is originally produced from two possible endmembers; denitrification/nitrifier-denitrification (denoted by subscript den) and nitrification/fungal denitrification (denoted by subscript nit). In each model we used identical SP endmember values (SP_{den} and SP_{nit}) and N₂O reduction isotope effects (εSP_{red} and ε¹⁸O_{red}) as those compiled in (Lewicka-Szczebak et al., 2017) (Table 2). For the δ¹⁸O-N₂O_{den}, the value used in Lewicka-Szczebak et al. (2017) was originally reported relative to the δ¹⁸O-H₂O (as δ¹⁸O-N₂O(N₂O/H₂O)). As we did not measure δ¹⁸O-H₂O in our samples, we reported and used our sample δ¹⁸O-N₂O values as is and then corrected the denitrification isotope signature, δ¹⁸O-N₂O(N₂O/H₂O)_{den}, reported by Lewicka-Szczebak et al. (2017) by an assumed δ¹⁸O-H₂O of water for our site. We used a δ¹⁸O-H₂O value of -8.3‰, as reported by Rapti-Caputo and Martinelli (2009) for an uncontaminated aquifer of the Po River delta. For the δ¹⁸O-N₂O_{nit}, we re-calculated the mean from the six studies used in Lewicka-Szczebak et al. (2017), using the original values reported as δ¹⁸O-N₂O (as opposed to δ¹⁸O-(N₂O/H₂O), this yielded a mean of 36.5‰ (Sutka et al., 2006; Sutka et al., 2008; Frame and Casciotti, 2010; Heil et al., 2014; Rohe et al., 2014; Maeda et al., 2015).
Closed system fractionation for N$_2$O reduction was modeled following the method described in Lewicka-Szczebak et al. (2017) (Fig.1). Here, sample SP and $\delta^{18}$O-N$_2$O values are used to derive sample specific intercepts that pass through the sample and reduction line (sc1) or the sample and the mixing line (sc2). A fixed slope for the reduction line can be calculated from $\varepsilon_{SP_{red}} / \varepsilon_{N_2O}^{18}$O (i.e. in our case, -5/-15). In sc1, the intercept of the mixing and reduction line represents N$_2$O that has been produced from denitrification/nitrifier-denitrification and partially reduced but not yet mixed with N$_2$O produced from nitrification/fungal denitrification. In sc2, the intercept of these lines represents N$_2$O that has been produced by the two endmember pools, mixed, but not yet reduced. The Y axis (i.e. SP) value of these respective intercepts can be used in a generalized Rayleigh equation (Eq. 4) to calculate the extent of N$_2$O reduction, represented by the fraction of residual N$_2$O not reduced.

$$SP_{resid:N_2O} \approx SP_{N_2O-unreduced} + \varepsilon_{SP_{red}} \cdot \ln(rN_2O_{net})$$  \hspace{1cm} (4)$$

In sc1 the $rN_2O$ is determined with respect to N$_2$O from denitrification/nitrifier-denitrification only, therefore to calculate the residual fraction of total production (i.e. N$_2$ + N$_2$O) we calculate gross $rN_2O$:

$$\text{gross \hspace{0.1cm} gross \hspace{0.1cm} rN_2O_{sc1} = \frac{1}{frac\text{Denit}_{net}/rN_2O_{net} + 1-frac\text{Denit}_{net}} \hspace{0.1cm} (sc1 \hspace{0.1cm} in \hspace{0.1cm} sc2 \hspace{0.1cm} rN_2O_{net} = rN_2O_{gross})$$ \hspace{1cm} (5)$$

To calculate the fraction of denitrification of the total initially produced N$_2$O (emitted as N$_2$O and N$_2$) we calculate the gross denitrification fraction:

$$\text{gross \hspace{0.1cm} fracDenit_{sc1-closed} = \frac{frac\text{Denit}_{net}/rN_2O_{net}}{frac\text{Denit}_{net}/rN_2O_{net} + 1-frac\text{Denit}_{net}} \hspace{0.1cm} (sc1)$$ \hspace{1cm} (6)$$

To calculate the fraction of denitrification/nitrifier-denitrification to the net N$_2$O produced, we use Eq. 7. For simplicity and comparison with open system calculations, we call this DenContribution.

$$\text{net \hspace{0.1cm} fracDenit}_{sc1-closed} = \frac{SP_{Net}}{SP_{Net}^n} \hspace{0.1cm} (sc1) \hspace{0.1cm} = \text{DenContribution}_{closed-sc1}$$ \hspace{1cm} (7)$$

In this case, $SP_{resid:N_2O}$ is the signature of residual bacterial N$_2$O after partial reduction but before mixing. This was determined from the graphical method (Lewicka-Szczebak et al., 2017). In sc2 both net and gross fractions of denitrification are equal and can be expressed as:

$$\text{DenContribution}_{closed-sc2} = \frac{SP_{Net}^n}{SP_{Net}^d} \hspace{0.1cm} (sc2)$$ \hspace{1cm} (8)$$

Here, $SP_{Net}^n$ is the signature of N$_2$O mixed from nitrification/fungal denitrification and denitrification/nitrifier-denitrification, but before reduction. This was determined from the graphical method (Lewicka-Szczebak et al., 2017).

To predict $rN_2O$ in open systems we set up a series of mass balance equations using our measured N$_2$O flux or N$_2O_{poreair}$ concentrations and measured $\delta^{18}$O and SP values. We used the same endmember values listed in Table 2 for all equations. As above, we can model the interaction between mixing and reduction assuming sc1 (Eqs 9-11) or sc2 (Eqs 9,12,13). In Eqs 9-13, we use $k_{nit}$, $k_{den}$ and $k_{red}$ to represent the gross process rates or concentrations of N$_2$O attributable to nitrification, denitrification and N$_2$O reduction, respectively.
\[ N_2O_{flux} \text{ (or } N_2O_{poreair} \text{)} = k_{nit} + k_{den} - k_{red} \]
\[ \text{note: } k_{den} = \text{total denitrification (N}_2\text{O + N}_2 \text{)} \] (9)

\[ SP - N_2O_{measured} = \frac{SP_{nit}k_{nit} + SP_{den} - \epsilon SP_{red}}{k_{nit} + k_{den} - k_{red}} \] (sc1)
\[ \delta^{18}O - N_2O_{measured} = \frac{(\delta^{18}ON_2O_{nit})k_{nit} + (\delta^{18}ON_2O_{den} - \epsilon \delta^{18}O_{red})k_{den}}{k_{nit} + k_{den} - k_{red}} \] (sc1)

\[ SP - N_2O_{measured} = \frac{(SP_{nit}k_{nit} + SP_{den}k_{den})}{k_{nit} + k_{den} - k_{red}} - \epsilon SP_{red} \left(1 - \frac{N_2O_{flux}}{k_{nit} + k_{den}}\right) \] (sc2)

\[ \delta^{18}O - N_2O_{measured} = \frac{(\delta^{18}ON_2O_{nit})k_{nit} + (\delta^{18}ON_2O_{den})k_{den}}{k_{nit} + k_{den} - k_{red}} - \epsilon \delta^{18}O_{red} \left(1 - \frac{N_2O_{flux}}{k_{nit} + k_{den}}\right) \] (sc2)

These two sets of equations (Eq. 9,10,11) or (Eq. 9,12,13), representing each scenario, were applied to measured surface fluxes to produce process rates in g N$_2$O-N ha$^{-1}$ d$^{-1}$ or were applied to N$_2$O$_{poreair}$ concentrations to produce concentrations of N$_2$O in μg N$_2$O-N L$^{-1}$. By rearranging these process rates or concentrations we can calculate gross rN$_2$O, frac$_{DEN}$ and the contribution of denitrification to N$_2$O using Eqs. 14-16.

\[ \text{gross frac}_{DEN \text{ sc1 sc2 - open}} = \frac{k_{den}}{k_{nit} + k_{den}} \] (14)
\[ \text{gross rN}_2\text{O}_{sc1 sc2 - open} = \frac{k_{nit} + k_{den} - k_{red}}{k_{nit} + k_{den}} \] (15)
\[ \text{DenContribution}_{sc1 sc1 - open} = \frac{(k_{den} - k_{red})}{[N_2O]} \text{, } [N_2O] = \text{N}_2\text{O}_\text{flux or N}_2\text{O}_\text{poreair} \] (16)

Plausible solutions for $k_{red}$, $k_{den}$, and $k_{red}$ were estimated based on minimizing the sum of squares between the modeled and measured N$_2$O flux (or concentration), $\delta^{18}$O and SP values using a Generalized Reduced Gradient (GRG) nonlinear algorithm in the Solver function of excel. Solutions with a minimum sum of squares over 500 were considered implausible (8.3% of solutions) (Table S2). Both models produced some non-plausible solutions, i.e. fractional contributions over 1 or under 0. Only solutions with a gross rN$_2$O, gross frac$_{DEN}$ and DenContribution between 0 to 1 and an open system minimum sum of squares < 500 were retained. In sc1, roughly 75% of solutions met these criteria. For sc2, less than 10% of solutions in the open system met this criteria, therefore we do not proceed to analyze and discuss solutions from sc2 (Table S2 and Fig. S3).

### 2.8 Statistical analyses

Response variables were analyzed using a linear mixed effects ANCOVA model with treatment, date, and depth (if applicable) as fixed effects and plot as a random effect. The longitudinal position in the field (Y position) measured in meters from the central driveway (Fig. S2), was used as a covariate to account for potential heterogeneity in the longitudinal direction. In the case of non-normally distributed data, data was transformed to obtain a normal distribution of residuals. Due to the non-normal distribution of many variables, Spearman correlations were used to analyze the relationship between N$_2$O$_{emitted}$ fluxes, isotopocule values, soil environmental and substrate variables. Post-hoc analysis of treatment and depth within a given day was performed using the lsmeans function with a Tukey adjustment for multiple comparisons. For the analysis of modeling results we eliminated the 25 cm depth due to poor data availability. All data analysis was done in R version 3.3.2.
3 Results

3.1 N\textsubscript{2}O fluxes, dissolved and pore air N\textsubscript{2}O concentrations

3.1.1 Temporal patterns in N\textsubscript{2}O fluxes and concentrations

After the first basal fertilization (May 16\textsuperscript{th}) and prior to the second topdressing fertilization (June 21\textsuperscript{st}), emissions were significantly higher in the DS-AWD treatment than in WS-AWD and WS-FLD on eight and six of the 11 sampling days, respectively (Fig. 2). During this time four peaks in emissions were observed in the DS-AWD treatment, on May 20\textsuperscript{th}, June 1\textsuperscript{st}-3\textsuperscript{rd}, June 7-9\textsuperscript{th}, and June 20\textsuperscript{th}, averaging 39.5 ± 5.1 g N\textsubscript{2}O-N ha\textsuperscript{-1} d\textsuperscript{-1}. A peak in emissions following the second fertilization (June 21\textsuperscript{st}) was observed in all treatments; in the DS-AWD treatment emissions peaked at 108.2 ± 4.2 g N\textsubscript{2}O-N ha\textsuperscript{-1} d\textsuperscript{-1} on June 23\textsuperscript{rd}, while in the WS-AWD and WS-FLD treatments, emissions peaked one day earlier reaching 49.4 ± 17.9 and 77.67 ± 10.6 g N\textsubscript{2}O-N ha\textsuperscript{-1} d\textsuperscript{-1}, respectively. In the WS-AWD treatment, emissions remained slightly elevated following this fertilization until the end of the monitoring campaign, while in the DS-AWD and WS-FLD, emissions declined after June 22 or 23\textsuperscript{rd}, respectively.

If we exclude N\textsubscript{2}O\textsubscript{dissolved} measurements from the DS-AWD treatment following the second fertilization (i.e. after the 22\textsuperscript{nd} of June, when concentrations reached as high as 594.4 ± 112.6 \(\mu\)g N\textsubscript{2}O-N L\textsuperscript{-1} at 5 cm), concentrations throughout the profile of all treatments remained under 20 \(\mu\)g N\textsubscript{2}O-N L\textsuperscript{-1}. Due to the large differences between dates and treatments we present the concentrations on a log\textsubscript{10} scale (Fig. 2) and non-transformed scale (Fig. S4). Peak concentrations in the WS treatments occurred at 5 cm on the first day of measurement, reaching 17.7 ± 5.1 and 18.5 ± 2.8 \(\mu\)g N\textsubscript{2}O-N L\textsuperscript{-1} in the WS-AWD and WS-FLD, respectively. In comparison, in the DS-AWD treatment peak concentrations prior to the second fertilization were observed at 25 cm on June 3\textsuperscript{rd}, reaching 18.5 ± 8.3 \(\mu\)g N\textsubscript{2}O-N L\textsuperscript{-1}.

As with dissolved N\textsubscript{2}O, pore air N\textsubscript{2}O concentrations were highly variable between treatments and between sampling days and are again presented on a log\textsubscript{10} scale (Fig. 2) and non-transformed scale (Fig. S4). In both WS treatments, the highest concentrations were observed on the first day of measurement, May 20\textsuperscript{th}, reaching 2903.3 ± 1103.6 and 1321 ± 998.0 \(\mu\)g N\textsubscript{2}O-N L\textsuperscript{-1} at 5 cm in the WS-FLD and WS-AWD, respectively. Elevated concentrations of N\textsubscript{2}O\textsubscript{poreair} were also observed in the DS-AWD on the first day of measurement but were 70.1 \(\mu\)g N\textsubscript{2}O-N L\textsuperscript{-1} at 5 cm (roughly 40x lower than in WS-FLD on this date). Maximum concentrations in the DS-AWD treatment were observed two days after the second fertilizer application, reaching 1902.2 \(\mu\)g N\textsubscript{2}O-N L\textsuperscript{-1}; in contrast no change was observed in the WS treatments following this fertilizer application. In all treatments the majority of N\textsubscript{2}O\textsubscript{poreair} concentrations were orders of magnitude lower than these peaks. There was a tendency of lower N\textsubscript{2}O\textsubscript{poreair} concentrations in the DS-AWD treatment relative to the WS treatments; this pattern was most evident at 5 cm (Fig. 2). However, treatment differences in N\textsubscript{2}O\textsubscript{poreair} were not significant (p=0.08, Table S3) and there was a significant date x treatment interaction.
3.1.2 Relation of $\text{N}_2\text{O}$ fluxes and concentrations with soil environment, substrates and $\text{N}_2\text{O}$ isotopocules

We evaluated the correlation of $\text{N}_2\text{O}_{\text{emitted}}$ with Eh, WFPS, NO$_3^-$, NH$_4^+$, dissolved and pore air $\text{N}_2\text{O}$ concentrations and $\text{N}_2\text{O}$ isotopocule ratios at 5 cm (Table 3). Among these variables, $\text{N}_2\text{O}$ emissions in the WS treatments were negatively correlated with pore water NH$_4^+$ and DOC in the WS-AWD treatment. In the DS-AWD treatment, emissions positively correlated with $\text{N}_2\text{O}_{\text{poreair}}$, WFPS, and NO$_3^-$ and negatively with $\text{N}_2\text{O}$ isotopocule signatures. Examining the isotopocule signatures of $\text{N}_2\text{O}_{\text{emitted}}$, we observed that $\text{N}_2\text{O}_{\text{emitted}}$ was negatively correlated with $\delta^{18}\text{O}-\text{N}_2\text{O}_{\text{emitted}}$ in all treatments, negatively with $\delta^{15}\text{N}-\text{N}_2\text{O}_{\text{emitted}}$ in the DS-AWD treatment and negatively with SP-$\text{N}_2\text{O}_{\text{emitted}}$ in the WS-FLD and DS-AWD. Interestingly, a positive correlation between $\text{N}_2\text{O}_{\text{emitted}}$ and SP-$\text{N}_2\text{O}_{\text{emitted}}$ was observed in the WS-AWD treatment. Relative to the DS-AWD, the WS treatments had fewer significant correlations between $\text{N}_2\text{O}$ isotopocules, soil environment or pore air $\text{N}_2\text{O}$ isotopocule signatures. DOC was positively correlated with $\delta^{15}\text{N}-\text{N}_2\text{O}_{\text{emitted}}$ in the WS-AWD and with $\delta^{18}\text{O}-\text{N}_2\text{O}_{\text{emitted}}$ in the WS-FLD. SP-$\text{N}_2\text{O}_{\text{emitted}}$ was positively correlated to Eh and negatively to WFPS in the WS-AWD treatment. In comparison, in the DS-AWD treatment, $\text{N}_2\text{O}$ isotopocule signatures of $\text{N}_2\text{O}_{\text{emitted}}$ were positively correlated to that of $\text{N}_2\text{O}_{\text{poreair}}$ for all three isotopocules. Furthermore, $\text{N}_2\text{O}$ isotopocule signatures in the DS-AWD treatment were negatively correlated with $\text{N}_2\text{O}_{\text{poreair}}$ concentrations, WFPS, NO$_3^-$ ($\delta^{15}\text{N}-\text{N}_2\text{O}_{\text{emitted}}$ only) and $\text{N}_2\text{O}_{\text{dissolved}}$ ($\delta^{18}\text{O}-\text{N}_2\text{O}_{\text{emitted}}$ and SP-$\text{N}_2\text{O}_{\text{emitted}}$ only). It should be noted that $\text{N}_2\text{O}_{\text{dissolved}}$ in the DS-AWD treatment was not measurable at the 5 cm depth on 10 of the 16 sampling dates due to low soil moisture and low pore water volumes.

3.2 Spatiotemporal patterns of $\text{N}_2\text{O}$ isotopocules

3.2.1 $\delta^{15}\text{N}-\text{N}_2\text{O}$

The $\delta^{15}\text{N}$ signatures of $\text{N}_2\text{O}_{\text{emitted}}$ showed high temporal variation across all treatments, while $\delta^{15}\text{N}-\text{N}_2\text{O}_{\text{poreair}}$ signatures changed less between sample dates and more discernable patterns across time could be seen (Fig. 3). A consistent temporal pattern of higher $\text{N}_2\text{O}_{\text{poreair}}$ concentrations and $\text{N}_2\text{O}_{\text{emitted}}$ fluxes in association with lower $\delta^{15}\text{N}$ signatures was observed in the DS-AWD treatment. In the WS treatments, high $\text{N}_2\text{O}_{\text{emitted}}$ fluxes were also associated with lower $\delta^{15}\text{N}$ signatures. $\text{N}_2\text{O}_{\text{poreair}}$ at 5 cm in the WS-AWD treatment tended to be higher in concentration and lower in $\delta^{15}\text{N}$ relative to other depths, however, in general a consistent relationship between concentration and $\delta^{15}\text{N}$ signatures was less evident in the two WS treatments. On average, the $\delta^{15}\text{N}$ signature of $\text{N}_2\text{O}_{\text{emitted}}$ was lower relative to $\text{N}_2\text{O}_{\text{poreair}}$ in the DS-AWD treatment. In contrast, in the WS treatments $\text{N}_2\text{O}_{\text{emitted}}$ was depleted in $^{15}\text{N}$ relative to $\text{N}_2\text{O}_{\text{poreair}}$ at all depths only immediately before and after the second fertilization. In these treatments, $\delta^{15}\text{N}-\text{N}_2\text{O}_{\text{poreair}}$ was generally lower at 5 cm relative the other depths but tended to increase and reach similar values as the other depths over the experimental period. As a result, $\text{N}_2\text{O}_{\text{emitted}}$ was often enriched in $^{15}\text{N}$ relative to $\text{N}_2\text{O}_{\text{poreair}}$ at 5 cm in these treatments, particularly in the WS-AWD treatment.
3.2.2 δ\textsuperscript{18}O-N\textsubscript{2}O

As with δ\textsuperscript{15}N, δ\textsuperscript{18}O signatures spanned a large range, particularly in the emitted N\textsubscript{2}O (Fig. 3). δ\textsuperscript{18}O-N\textsubscript{2}O\textsubscript{poreair} in the DS-AWD followed a temporal pattern similar to δ\textsuperscript{15}N signatures and similarly, δ\textsuperscript{18}O signatures were generally lower in N\textsubscript{2}O\textsubscript{emitted} relative to N\textsubscript{2}O\textsubscript{poreair}. The highest δ\textsuperscript{18}O-N\textsubscript{2}O\textsubscript{poreair} was seen in the DS-AWD treatment at moderate N\textsubscript{2}O\textsubscript{poreair} concentrations where δ\textsuperscript{18}O signatures were higher than other concentrations in the DS-AWD or any concentration in the WS treatments. These samples were also nearly always taken from 12.5 or 25 cm. In all treatments, lower δ\textsuperscript{18}O signatures were observed in N\textsubscript{2}O\textsubscript{poreair} and N\textsubscript{2}O\textsubscript{emitted} on the first day of sampling, global mean of 35.1 ± 1.1 and 29.6 ± 1.7‰ relative to 46.9 ± 0.4 and 43.9 ± 1.7‰, respectively. Otherwise, no distinct pattern with depth, time, or concentration was observed in the WS treatments.

3.2.3 SP-N\textsubscript{2}O

The SP of N\textsubscript{2}O\textsubscript{emitted} ranged from 4.5 ± 0.4 to 25.6 ± 8.1‰, from 2.9 ± 1.0 to 37.2‰ (un-replicated) and from 5.8 ± 0.6 to 40.6 ± 12.4‰, in the DS-AWD, WS-AWD, and WS-FLD treatments, respectively (Fig. 3). In contrast to δ\textsuperscript{15}N and δ\textsuperscript{18}O signatures, the SP-N\textsubscript{2}O\textsubscript{poreair} tended to increase with time, but only in the WS treatments. As with δ\textsuperscript{15}N-N\textsubscript{2}O and δ\textsuperscript{18}O-N\textsubscript{2}O, moderate and lower concentration N\textsubscript{2}O\textsubscript{poreair} samples showed higher SP values relative to higher concentration N\textsubscript{2}O\textsubscript{poreair} samples. For example, two days after the second fertilizer application (June 23\textsuperscript{rd}), SP values decreased in conjunction with increased N\textsubscript{2}O\textsubscript{poreair} concentrations in the DS-AWD treatment. On this date mean SP values at 5 cm demonstrated the largest treatment differences with values of: 0.7 ± 4.5, 27.6 ± 2.1, and 39.9 ± 2.7‰ in the DS-AWD, WS-AWD, and WS-FLD treatments, respectively. On this date, the pattern between the treatments was consistent throughout the three depths.

3.2.4 Relationships between N\textsubscript{2}O isotopocules

Considering all depths and emitted data together, δ\textsuperscript{18}O-N\textsubscript{2}O signatures significantly and positively correlated with δ\textsuperscript{15}N-N\textsubscript{2}O and SP across all treatments. The slope of δ\textsuperscript{18}O-N\textsubscript{2}O vs. δ\textsuperscript{15}N-N\textsubscript{2}O was 0.67, 0.28, and 0.52 (Fig. S5) and 0.67, 0.54 and 0.31 for SP vs. δ\textsuperscript{18}O-N\textsubscript{2}O in the DS-AWD, WS-AWD, and WS-FLD treatments, respectively (Fig. 4a). There was no correlation between SP and δ\textsuperscript{15}N-N\textsubscript{2}O in the two WS treatments, but a positive correlation for the DS-AWD was found, with a slope of 0.62 (Fig. 4b). Examining these relationships by depth, we saw the strongest relationship and highest slope in the N\textsubscript{2}O\textsubscript{emitted} and at 25 cm for δ\textsuperscript{18}O-N\textsubscript{2}O vs δ\textsuperscript{15}N-N\textsubscript{2}O (Fig. S5). While the SP vs δ\textsuperscript{18}O-N\textsubscript{2}O showed no correlation among the surface fluxes in the WS treatments, the two isotopocules were positively correlated in N\textsubscript{2}O\textsubscript{poreair} at all depths and treatments (Fig. S6). A contrasting relationship between SP and δ\textsuperscript{15}N-N\textsubscript{2}O was observed for the WS-FLD treatment in the N\textsubscript{2}O\textsubscript{emitted} and N\textsubscript{2}O\textsubscript{poreair} where the two isotopocules were negatively correlated in N\textsubscript{2}O\textsubscript{emitted} and positively in N\textsubscript{2}O\textsubscript{poreair} (Fig. S7).
3.3 NO\textsubscript{3} and NH\textsubscript{4}+ concentrations and isotope signatures

3.3.1 Spatiotemporal trend in NO\textsubscript{3} and NH\textsubscript{4}+ concentration and δ\textsuperscript{15}N and δ\textsuperscript{18}O signatures

In all treatments, pore water NH\textsubscript{4}+ concentrations were highest at 5 cm relative to the other depths (Fig. 2). In the DS-AWD treatment concentrations were almost null prior to the second fertilization, remaining below 0.85 mg NH\textsubscript{4}+-N L\textsuperscript{-1} across all depths. Following this fertilization, concentrations increased at all depths, most notably at 5 cm. An opposing pattern was observed in the WS treatments where NH\textsubscript{4}+ was nearly always significantly higher than in DS-AWD for each corresponding depth leading up to the second fertilization, but dropped to near zero following the fertilization. Nitrate concentrations were exclusively less than 1.5 mg NO\textsubscript{3}-N L\textsuperscript{-1} in both WS treatments throughout the experimental period. In sharp contrast, NO\textsubscript{3} concentrations in the DS-AWD were at times more than 75 times higher than in WS treatments, peaking on June 1st at 113.6 ± 22.4 mg NO\textsubscript{3}-N L\textsuperscript{-1}. Following this spike, concentrations steadily declined and dropped to null following the second fertilization.

3.3.2 δ\textsuperscript{15}N-NO\textsubscript{3}, δ\textsuperscript{15}N-NH\textsubscript{4}+ and isotope enrichment factors: ε\textsuperscript{15}N\textsubscript{N2O/NO3} and ε\textsuperscript{15}N\textsubscript{N2O/NH4}

Concentrations of NO\textsubscript{3} or NH\textsubscript{4}+ were often too low for isotope measurements. Hence, we could only obtain sufficient replication for statistical analysis across depths and treatments on five days for NO\textsubscript{3} (May 24\textsuperscript{th}, 27\textsuperscript{th}, June 1\textsuperscript{st}, 14\textsuperscript{th}, 23\textsuperscript{rd}) and two days for NH\textsubscript{4}+ (May 24\textsuperscript{th} and June 23\textsuperscript{rd}) (Fig. S9). Daily mean δ\textsuperscript{15}N-NO\textsubscript{3} ranged from -4.3 to 28.3‰ across all treatments and depths. In the DS-AWD treatment a consistent depth pattern was observed with 15N enrichment of NO\textsubscript{3} at 25 cm > 12.5 cm = 5 cm. δ\textsuperscript{15}N-NO\textsubscript{3} signatures increased with time at 5 cm, rising from −4.3 ± 1.5‰ to 22.0 ± 4.9‰. Significant treatment and depth differences were observed on May 24\textsuperscript{th}, 27\textsuperscript{th} and June 1\textsuperscript{st}, but no differences were observed on later dates, June 14\textsuperscript{th} or 23\textsuperscript{rd}. Following the second fertilizer application, δ\textsuperscript{15}N-NO\textsubscript{3} signatures in the DS-AWD treatment rose by approximately 10‰ at all depths. Daily mean δ\textsuperscript{15}N-NH\textsubscript{4}+ ranged from -6‰ to 15.2‰ (Fig. S9). Averaging across the experimental period and depths, mean δ\textsuperscript{15}N signatures of NO\textsubscript{3} and NH\textsubscript{4}+ were similar, 8.4 and 7.0‰, respectively (Table S5). There was no evident temporal or depth trend in δ\textsuperscript{15}N-NH\textsubscript{4}+ in any of the treatments. The only significant difference was lower δ\textsuperscript{15}N-NH\textsubscript{4}+ in the DS-AWD on June 23\textsuperscript{rd}. δ\textsuperscript{15}N-NO\textsubscript{3} values positively correlated to N\textsubscript{2}O\textsubscript{poreair} concentrations in the DS-AWD and WS-FLD treatments and were negatively correlated to NO\textsubscript{3} concentrations and to δ\textsuperscript{15}N-NH\textsubscript{4}+ in the DS-AWD treatment (Table 4). δ\textsuperscript{15}N-NH\textsubscript{4}+ was negatively correlated to N\textsubscript{2}O\textsubscript{poreair} concentrations and NH\textsubscript{4}+ concentrations and positively to δ\textsuperscript{15}N-N\textsubscript{2}O\textsubscript{poreair} in the DS-AWD treatment.

Largely reflecting the depth pattern of δ\textsuperscript{15}N-NO\textsubscript{3} in the DS-AWD, the calculated ε\textsuperscript{15}N\textsubscript{N2O/NO3} tended to be highest at 5 cm, mean -7.2 ± 2.7‰, while mean values at 12.5 and 25 cm were slightly lower, -9.5 ± 2.0 and -16.0 ± 2.1‰, respectively (Fig. S9). At 5 cm ε\textsuperscript{15}N\textsubscript{N2O/NO3} values in the DS-AWD were significantly higher than in the WS treatments; at 12.5cm they tended to be higher as well but the difference was not significant. Two days after the second fertilizer application, the ε\textsuperscript{15}N\textsubscript{N2O/NO3} in the DS-AWD markedly decreased at all depths to a treatment mean of -23.6 ± 2.6‰. In comparison, WS treatment ε\textsuperscript{15}N\textsubscript{N2O/NO3}
values rose one (WS-FLD) or two (WS-AWD) days following the fertilization. In the WS-FLD, the increase in $\delta^{15}$N$_{\text{N2O/NO3}}$ values lasted only one day; unfortunately low NO$_3^-$ concentrations precluded $\delta^{15}$N-NO$_3^-$ analysis on many dates making temporal patterns difficult to observe. Mean depth by treatment isotope effects calculated relative to $\delta^{15}$N-NH$_4^+$ ($\epsilon^{15}$N$_{\text{N2O/NH4}}$) were $-12.7 \pm 3.2\%$, $-24.5 \pm 2.6\%$ and $-20.6 \pm 2.2\%$ at 5 cm; $-9.9 \pm 4.0\%$, $-12.8 \pm 2.8\%$ and $-15.9 \pm 1.9\%$ at 12.5 cm; $-17.0 \pm 5.9\%$, $-6.4 \pm 1.7\%$ and $-5.8 \pm 2.7\%$ at 25 cm for DS-AWD, WD-AWD and WD-FLD, respectively. Data for $\epsilon^{15}$N$_{\text{N2O/NH4}}$ was scarce in the DS-AWD treatment due to low NH$_4^+$ concentrations, in the WS treatments $\epsilon^{15}$N$_{\text{N2O/NH4}}$ increased with depth, but these differences were not significant.

$\delta^{18}$O-NO$_3^-$ was significantly depleted in the DS-AWD treatment relative to both WS treatments (Fig. S9). Prior to the second fertilization, values were remarkably consistent in the DS-AWD at all depths, ranging from 0.1 to 7.5\%. Two days after this fertilizer application, $\delta^{18}$O-NO$_3^-$ rose to a mean of 7.6\% across depths. In comparison the $\delta^{18}$O-NO$_3^-$ of both WS treatments was more variable between sampling dates, fluctuating between 12.2 to 38.8 and 10.4 to 32.7\% leading up the second fertilization in the WS-AWD and WS-FLD, respectively. Two days after the second fertilizer application values rose to a mean of 23.7 and 27.4\% across depths in the WS-AWD and WS-FLD, respectively. We calculated the net isotope effect for $\delta^{18}$O relative to water ($\epsilon^{18}$O$_{\text{N2O/H2O}}$). The $\epsilon^{18}$O$_{\text{N2O/H2O}}$ in all treatments and depths tended to rise over the course of the measurement period, with the most consistent rise observed at 5 cm. Here values rose from a global mean of 43.8 $\pm$ 1.0\% on May 20th to 58.5 $\pm$ 1.0\% on June 30th. There was a pattern of higher $\epsilon^{18}$O$_{\text{N2O/H2O}}$ in the DS-AWD treatment relative to the two WS treatments. A drop in $\epsilon^{18}$O$_{\text{N2O/H2O}}$ of $\sim$ 10\% was observed in all depths on June 23rd, two days after the second fertilization with urea, in the DS-AWD only.

3.4 SP x $\delta^{18}$O-N2O two endmember mixing model to estimate N2O reduction, source contributions, and N2O reduction

To further quantitatively interpret our isotopocule data, we employed a graphical two end-member mixing model (Lewicka-Szczech et al., 2017), based on the relationship between SP and $\delta^{18}$O-N$_2$O (Fig. 1 and 4). Data was modeled for open and closed fraction dynamics under two scenarios. In sc1 reduction of N$_2$O from the denitrification/nitrifier-denitrification endmember pool occurs prior to mixing with nitrification/fungal denitrification derived N$_2$O; in sc2, mixing of N$_2$O from both endmember pools occurs before reduction. For sc2 our model yielded implausible results for the contribution of denitrification/nitrifier-denitrification to N$_2$O emissions in about 90% and 20% of observations under open and closed system dynamics, respectively (Table S2). The poorer outcomes from sc2 in the open system indicate that the assumptions underlying this scenario are likely false in open systems or vice versa. In order to have comparable data between open and closed systems we discuss only results coming from sc1 simulations.

Temporal trends in the gross rates of rN$_2$O (extent of N$_2$O reduction) predicted by open and closed system N$_2$O fractionation were nearly identical (Fig. 5b). Gross rN$_2$O was estimated to be higher (i.e. lower N$_2$O reduction) under closed system fractionation dynamics. In reality, it can be assumed that neither perfect open or closed systems exist in nature and processes
likely reflect a mixture of these dynamics. The use of one or the other case may bias results, therefore we chose to take the mean of the two systems to estimate N₂O reduction, nitrification/fungal denitrification and denitrification/nitrifier-denitrification derived N₂O emissions (Decock and Six, 2013b; Wu et al., 2016). Due to a disproportionate number of missing values at 25 cm in the two WS treatments, we chose not to include data from this depth in our analysis and discussion. Therefore, further values refer to the mean of open and closed systems and N₂O_{\text{emitted}} or N₂O_{\text{poreair}} at 5 cm and 12.5 cm unless explicitly stated otherwise. Gross rN₂O fractions tended to be higher in N₂O_{\text{emitted}} (treatment means 0.14 to 0.19) relative to the subsurface (treatment means 0.06 to 0.15). While water management treatment had a significant effect on process contributions to N₂O_{\text{emitted}} and N₂O_{\text{poreair}} (Table 5), significant interactions with depth and date were observed. Gross rN₂O fractions in N₂O_{\text{poreair}} were significantly lower in the DS-AWD relative to the WS-FLD on six of 15 days, with the WS-AWD falling in between. In the N₂O_{\text{emitted}}, the opposite pattern was mostly observed with gross rN₂O fractions often being higher in the DS-AWD than one or the other WS treatments, significantly so on four of 15 days. Aggregated across depths, the contribution of denitrification/nitrifier-denitrification to N₂O_{\text{poreair}} were higher in the DS-AWD relative to one or both WS treatments on four dates and lower on three dates (Fig. 5a). The mean contribution of denitrification/nitrifier-denitrification to N₂O_{\text{emitted}} ranged from 43 to 49% in all treatments (Fig. 6). Denitrification/nitrifier-denitrification contributions to N₂O_{\text{emitted}} were higher in the DS-AWD relative to the WS treatments on June 9th and 23rd and relative to WS-AWD only they were also higher on June 28th and lower on June 21st.

4 Discussion

4.1 Patterns of N₂O_{\text{emitted}}, N₂O_{\text{poreair}}, N₂O isotopocule ratios and net isotope effects

In accordance with results from past studies (Cai et al., 1997; Miniotti et al., 2016; Peyron et al., 2016) and in line with our hypothesis, we observed higher N₂O emissions on most days in the DS-AWD relative to the two WS treatments (Fig. 2). A belated divergence in water management between the WS-FLD and WS-AWD (Table 1), in addition to a relatively wet early summer, likely contributed to similar observed soil environmental conditions and N substrates among these two treatments. Therefore, given the similarities in soil conditions, it is not surprising that N₂O fluxes and isotopocule differences between these two treatments were generally fewer than expected. Mean daily δ¹⁵N, δ¹⁸O and SP values of N₂O_{\text{emitted}} and N₂O_{\text{poreair}} per depth and treatment ranged from -27.9 to 12.3‰, 30.9 to 63.0‰ and -14.0 to 53.2‰, respectively (Fig. 3). These values are similar in magnitude to those observed by Yano et al., (2014) in the early growing season of rice, where ranges of -24 to 6‰, 24 to 66‰ and 4 to 25‰ were reported. Our values are also similar in magnitude to those observed in other field studies which have included depth sampling (Koehler et al., 2012; Zou et al., 2014). Relative to these two studies we observed higher δ¹⁵N-N₂O and both higher and lower SP ratios. This was likely due to a higher sampling frequency, which covered more variable soil environments and generally higher soil moisture in our study than in the others. For example, it has been shown that organic matter decomposition and DOC availability in
rice systems can decline with the introduction of wet-dry cycles or dry seeding (Yao et al., 2011; Said-Pullicino et al., 2016); thus it is likely that conditions promoting complete denitrification declined in the AWD treatments. While, saturated conditions promoting complete denitrification may have a strong impact on isotope signatures. Working in a denitrifying aquifer, Well et al. (2012) observed very large ranges in δ15N and SP ratios, varying from -55.4 to 89.4‰ and 1.8 to 97.9‰, respectively.

The calculated ε15N2O3/N2O (net isotope effect) in the DS-AWD treatment, with depth means of -7.2 to -16.0‰, was consistently much higher (i.e. less strong fractionation) than literature values reported for denitrification of NO3-, mean: -42.9 ± 6.3‰ (Denk et al., 2017)(Fig. S9). At 5 cm in the two WS treatments, the mean ε15N2O3 was lower than in the DS-AWD (-23.2 and -21.5 in the WS-AWD and WS-FLD, respectively), but still nearly 20‰ higher than literature values. In a rice system, Yano et al. (2014) observed an ε15N2O3 of -6.7‰, thus very well within the range of our calculated ε15N2O3. Similarly, the global mean of our ε15N2O3 values was -14.8‰, thus on average much higher than those reported in the literature for nitrification, -46.9‰ (Sutka et al., 2006) or -56.6 ± 7.3‰ (Denk et al., 2017). For both isotope effects, similar scenarios may explain our high observed ε15N (i.e. low fractionation). Namely, i) non-steady state reactions, for example rapid refreshing of the NO3- and NH4+ pools or near complete substrate consumption or ii) significant reduction of N2O serving to increase δ15N-N2O values and thereby reduce the net isotope effect.

Considering the moist conditions and high reduction rates, it seems most likely that strong N2O reduction was the largest contributor to our high net isotope effects. To check this, we estimated initial δ15N-N2O values before N2O reduction using our modeled N2O reduction fraction (rN2O), measured δ15N-N2O values and a 15N isotope effect during reduction of -6.6‰ (Denk et al., 2017) in the Rayleigh equation. We could then estimate amended ε15N2O3 values if N2O reduction effects were accounted for, from the difference between our initial δ15N-N2O estimates and δ15N-NO3. These calculations yielded a ε15N2O3 from -25.0 to -36.5‰, -32.6 to -42.3‰ and -29.0 to -51.1‰ in the DS-AWD, WS-AWD and WS-FLD across depths (Table S6). These amended ε15N2O3 values do decrease and especially for the WS treatments, come relatively close to literature values for ε15N2O3 values during denitrification. Thus, significant N2O reduction can likely explain much of the high ε15N2O3 values observed, particularly in the WS treatments. Yet other factors were also likely at play to some degree. For example, in the DS-AWD, where we observed evidence of significant nitrification, it is quite possible to envision isolated enrichment of NO3- in anaerobic microsites where N2O is produced, while the bulk soil NO3- pool remained less enriched. It is also true that we could not always measure δ15N values of NO3- or NH4+ because the concentrations were too low, thus we could not calculate isotope effects. This highlights a persistent dilemma, which is true for all isotopocules, that we cannot accurately measure isotope values at very low concentrations. Hence, in situ measurements such as these will always be biased toward higher concentration scenarios where perhaps the strongest and most interesting effects of substrate enrichment are missed.
4.2 Source partitioning N₂O production

The use of any one isotope signature alone is confounded by overlap in the isotope effects between processes, unknown and possibly rapidly changing substrate δ values and the unknown contribution of N₂O reduction effects. To overcome these drawbacks, graphical interpretations of dual N₂O isotopocules have been used in field studies to interpret datasets similar to ours (Koehler et al., 2012; Well et al., 2012). For a more quantitative assessment of source-partitioning, mixing models using a dual isotope approach can be used (Koba et al., 2009; Toyoda et al., 2011; Yano et al., 2014; Zou et al., 2014; Lewicka-Szczebak et al., 2017). In the subsequent analysis we employ both approaches using our samples values plotted in SP x δ¹⁸O and SP x δ¹⁵N space (Fig. 4 and Figs. S10-S12).

In both SP x δ¹⁸O and SP x δ¹⁵N plots our sample values mostly fell between the mixing and reduction lines predicted by either isotopocule relationship (Fig. 4) and somewhat surprisingly showed a stronger trajectory towards N₂O reduction in the DS-AWD treatment relative to the WS treatments. In the DS-AWD and to a lesser extent in the WS-AWD treatment, high pore air N₂O concentrations were associated with denitrification or nitrifier-denitrification, while mid-range concentrations were associated with a higher degree of N₂O reduction and the lowest concentrations fell neatly in between. Similarly, in the WS-FLD treatment, denitrification or nitrifier-denitrification associated samples almost exclusively coincided with high N₂O_poreair. Most likely the moderate N₂O_poreair concentrations derived from N₂O reduction following high denitrification/nitrifier-denitrification production. This analysis is supported by data showing a trend of enrichment over the course of the measurement period (Fig. S10) and high WFPS values associated with the most enriched N₂O_poreair in the DS-AWD (Fig. S12). All treatments showed an enrichment of SP with time (Fig. S10), but interestingly only in the DS-AWD did δ¹⁸O and δ¹⁵N-N₂O enrich over the course of the experiment. This may reflect an increase over time in δ¹⁵N and δ¹⁸O of NO₃⁻, which was observed in the DS-AWD treatment, albeit not strongly (Fig. S9), yet one could expect a stronger enrichment of δ¹⁵N and δ¹⁸O-NO₃⁻ in denitrifying microsites.

We observed a scattering of high to moderate concentration N₂O_poreair values in the WS treatments that corresponded to higher SP values relative to δ¹⁸O or δ¹⁵N than would be expected by reduction enrichment (Fig. 4). We postulate that these values could be explained by greater contributions from abiotic hydroxylamine decomposition (SP ~ 34-35‰, Heil et al. (2014)) or fungal denitrification (SP ~ 35‰, Rohe et al. (2014)). Zhou et al. (2001) showed that fungal denitrification requires minimal oxygen to proceed, similarly Seo and DeLaune (2010) found that fungal denitrification dominated relative to bacterial denitrification at modest reducing conditions to weakly oxidizing conditions (Eh >250 mV). Indeed, there is some evidence that these high scattered SP values corresponded to more moderate WFPS (70-90%) in the WS-FLD treatment (Fig. S12). Abiotic hydroxylamine decomposition requires nitrification for the production of NH₃OH, and iron or manganese (hydr)oxides as electron acceptors to proceed (Bremner et al., 1980). These species can co-occur in the rhizosphere of a flooded rice soil, were O₂ is transported to the immediate root zone by the aerenchyma, for example, tightly coupled
nitrification-denitrification in the rhizosphere of rice plants has been shown before (Arth and Frenzel, 2000) as has coupling of nitrogen – iron transformations (Ratering and Schnell, 2000).

It is necessary to contextualize N\textsubscript{2}O isotopocule data with our measured substrate concentrations and soil environmental data. Based on our observations of low NH\textsubscript{4}\textsuperscript{+} concentrations, high NO\textsubscript{3}\textsuperscript{-} concentrations, an Eh over 400 mV and WFPS often below 60\% (5 cm) or below 85\% (12.5 and 25 cm) in the DS-AWD treatment, we can safely deduce that extensive nitrification of either basal urea fertilizer or of indigenous soil N occurred in this treatment (Fig. 2). Furthermore, the \( \delta^{18}\text{O}-\text{NO}_3\) in the DS-AWD treatment ranged from 0.1 to 14.8 (Fig. 7), thus falling in the range attributed to NO\textsubscript{3} \textsuperscript{-} produced from nitrification (Kendall and McDonnell, 2012). Additionally, we observed that both \( \delta^{15}\text{N}-\text{NO}_3 \) and \( \delta^{15}\text{N-NH}_4\textsuperscript{+} \) were negatively correlated to substrate concentrations in the DS-AWD treatment, indicative of active consumption of both N substrates (Table 4). In the DS-AWD, there also was a positive correlation between \( \delta^{15}\text{N}-\text{NO}_3 \) and \( \text{N}_2\text{O}_{\text{poreair}} \) but a negative correlation between \( \delta^{15}\text{N-NH}_4\textsuperscript{+} \) and \( \text{N}_2\text{O}_{\text{poreair}} \). The former likely indicates N\textsubscript{2}O production via denitrification and subsequent enrichment of the NO\textsubscript{3} \textsuperscript{-} pool. The latter is more difficult to interpret, but we attributed this to higher emissions associated with fresh inputs of NH\textsubscript{4}\textsuperscript{+} (from urea or mineralization) which should have a \( \delta^{15}\text{N} \) value around 0\%. Together this data shows that coupled nitrification-denitrification was responsible for the majority of N\textsubscript{2}O emissions. Similar results were also reported by Dong et al. (2012) for an AWD system. The separation of isotopocule signatures by date, N\textsubscript{2}O concentration and WFPS suggests that NO\textsubscript{3} \textsuperscript{-} produced early in the growing season was progressively denitrified and reduced over the course of the sampling period. Similarly, N\textsubscript{2}O produced early in the growing season may have been progressively reduced.

### 4.3 Inferring the extent of N\textsubscript{2}O reduction

It has been suggested that the slope of SP/\( \delta^{18}\text{O} \), SP/\( \delta^{15}\text{N} \) and \( \delta^{18}\text{O}/\delta^{15}\text{N} \) or their isotope effects can be used to estimate the extent of N\textsubscript{2}O reduction (Ostrom et al., 2007; Jinuntuya-Nortman et al., 2008; Well and Flessa, 2009; Lewicka-Szczebak et al., 2017). However, many studies deriving these relationships have taken place under controlled conditions when N\textsubscript{2}O supply was often limited. Therefore fractionation followed closed system dynamics would result in larger fractionation effects on the residual substrate than under open system dynamics. The positive and significant relationship between all isotopocules and across all depths in the DS-AWD treatment suggests an influence of reduction at all depths. In contrast, in the WS treatments we observed no relationship between SP and \( \delta^{18}\text{O} \) within N\textsubscript{2}O\textsuperscript{emitted} (Fig. S7) and only a weak relationship between SP and \( \delta^{15}\text{N} \) at 25 cm in the WS-AWD, and even a negative relationship between SP and \( \delta^{15}\text{N} \) in the WS-FLD N\textsubscript{2}O\textsuperscript{emitted} (Fig. S8). The range of observed \( \delta^{18}\text{O}/\delta^{15}\text{N} \) slopes, 0.21 to 0.90, (Fig. S5) were substantially lower than those observed in many N\textsubscript{2}O reduction studies (1.94 to 2.6; Jinuntuya-Nortman et al., 2008; Ostrom et al., 2007; Well and Flessa, 2009; Lewicka-Szczebak et al., 2017), but closer to the 0.45 slope observed by Yano et al. (2014) in an in situ rice field study. When a significant relationship was observed, overall or N\textsubscript{2}O\textsuperscript{poreair} SP/\( \delta^{15}\text{N} \) slopes ranged from 0.49 to 0.83 (Fig. 4b). These slopes are either close to those of other field studies, 0.48 to 0.52 (Yano et al., 2014; Wolf et al., 2015) or intermediary between field studies and controlled N\textsubscript{2}O reduction studies, 0.59 to 1.01 (Well and Flessa, 2009; Lewicka-Szczebak et al., 2017). From
controlled N₂O reduction studies, a SP/δ¹⁸O slope between 0.2 to 0.4 has been observed (Jinuntuya-Nortman et al., 2008; Well and Flessa, 2009), thus in this case the N₂O_poreair slopes observed in our study were substantially higher (Fig. 4a and Fig. S7). The lower overall SP and δ¹⁸O slope in the WS treatments was due to inclusion of the N₂O_emitted values, which individually showed no relationship in these treatments.

A deviation in slopes compared to those observed in controlled N₂O reduction studies likely points to a growing influence of open system dynamics where substrates are continuously refreshed. It has been demonstrated that when mixing processes dominate over reduction processes, the SP/δ¹⁸O slope rises (Lewicka-Szczebak et al., 2017). It is also plausible that high rates of oxygen exchange during denitrification served to partially mask an increase in δ¹⁸O-N₂O values, resulting in the higher observed SP/δ¹⁸O slopes or lower δ¹⁸O/δ¹⁵N slopes. To estimate the extent of oxygen exchange with denitrification precursors (NOx) we plotted δ¹⁸O-N₂O/δ¹⁸O-NO₃ by δ¹⁸O-H₂O/δ¹⁸O-NO₃ following (Snider et al., 2009). The slope of this relationship ranged from 0.7 to 2.1 (data not shown). Thus we assume oxygen exchange was effectively 100% across treatments during denitrification. In summary, the observed positive relationships between the isotopocule pairs is indicative of an influential role of N₂O reduction in the DS-AWD treatment. This is less clear in the WS treatments where relationships were more erratic, suggesting a stronger influence of changing nitrification and denitrification process rates or changing δ¹⁵N of N substrates. It is likely that isotope ratios in the WS treatments were affected by near complete denitrification to N₂. Well et al. (2012) observed highly variable isotopocule ratios in a strongly denitrifying aquifer and concluded that N₂O reduction was strongly progressed but variable. However, it should be noted that their system had abundant NO₃ while ours did not. The inconsistent relationships between N₂O_emitted and N₂O_poreair for SP/δ¹⁵N and SP/δ¹⁸O in the WS treatments and the stronger enrichment observed in the DS-AWD N₂O_emitted (Fig. 4) demonstrate a disconnection between subsurface N₂O_emitted and N₂O_poreair across treatments. Such results suggest that N₂O reduction may not have had as strong of an influence on the signature of N₂O_emitted as it did on N₂O_poreair, particularly in the WS treatments. A de-coupling between subsurface N₂O concentrations and surface emissions, and their isotopocule ratios has been observed in other studies (Van Groenigen et al., 2005; Goldberg et al., 2010a). This phenomenon is most simply explained by emitted N₂O truly coming from a mix of sources and depths, while subsurface N₂O is representative of a much smaller spatial zone and more likely to be dominated by one process. While difficult to practically measure, processes at shallow depths above 5 cm, were also likely influential to surface emissions.

4.4 Complementary evidence from a two endmember mixing model approach

To quantitatively estimate the extent of N₂O reduction (gross rN₂O), N₂O production and reduction rates, and the contribution of denitrification to N₂O emissions, we used an open and closed system two endmember mixing model based on SP-N₂O and δ¹⁸O-N₂O relationships. As described in section 2.7, we tested our models under two scenarios; in scenario one (sc1) N₂O is produced and reduced by denitrifiers before mixing with N₂O derived from nitrification, in scenario two (sc2) N₂O is produced from both processes, mixed, and then reduced (Fig. 1). While we could estimate gross rN₂O and the fraction of denitrification from both scenarios, sc2 yielded mostly implausible solutions for the contribution of denitrification to N₂O in open systems...
(Fig. S3 and Table S2). We thus conclude that the assumptions underlying this scenario in open systems were not valid in our system. In a closed system N₂O is progressively consumed and not replenished, resulting in a stronger isotope effect and faster enrichment of the remaining N₂O; thus a smaller degree of N₂O reduction is needed to achieve an equivalent enrichment as in open systems. Our results for open and closed systems align well with this theory on N₂O fractionation. Given the lower moisture and evidence of extensive nitrification occurring in the DS-AWD treatment, we expected a higher contribution of nitrification/fungal denitrification in this treatment, coming from an increase in nitrification. However, this was not the case and denitrification/nitrifier-denitrification contributions tended to be higher in the DS-AWD treatment relative to WS treatments (Fig. 5a, Fig. 6). Treatment differences were significant in the surface fluxes, however there was a significant interaction with sampling day; there was no treatment effect on denitrification contribution in the subsurface (Table 5). The equivalent or higher contributions of nitrification/fungal denitrification in the WS treatments (Fig. 6) are most easily explained by higher fungal denitrification; in their laboratory experiments, Lewicka-Szczebak et al. (2017) also observed relatively high fungal denitrification contributions under very wet conditions. Larger contributions from fungal denitrification would also help explain the less clear reduction trends as fungal denitrifiers are thought to largely produce N₂O as an end-product rather than N₂. It should be noted that due to low surface fluxes or N₂O_partial, we had fewer data points in the WS treatments. Previous studies have attributed significant amounts of N₂O emissions in paddy systems to nitrification in periods of low soil moisture (Lagomarsino et al., 2016; Verhoeven et al., 2018). Yet, such studies were not able to quantitatively source-partition emissions. Given our results here, it is possible that N₂O produced either via nitrifier-denitrification or coupled nitrification-denitrification has been previously underestimated.

The modeled gross N₂O fractions indicate high levels of N₂O reduction for all treatments and depths, (rN₂O: 0.06 to 0.19) even in the DS-AWD where soil moisture was frequently below 60% at 5 cm (Fig. 2). These results are at first surprising, but there is still much we do not know about subsurface N₂O production and consumption. Direct measurements of N₂O reduction at depth are few. Using membrane inlet mass spectrometry, Zhou et al. (2017) detected higher N₂O reduction to N₂ in paddy soil water at 20 cm versus 60 or 80 cm and could relate this to higher DOC concentrations at 20 cm. Other studies suggest high subsurface N₂O reduction based on the inference of declining N₂O concentration accompanied by isotope enrichment moving up a soil profile (Clough et al., 1998; Van Groenigen et al., 2005; Goldberg et al., 2008). We are also methodologically limited by our inability to measure N₂O isotopocules at near, or complete N₂O reduction because there is too little remaining N₂O to measure. We assume this was more often the case in the WS treatments, therefore we postulate that the signature of N₂O reduction was stronger in the DS-AWD largely because there was more N₂O left to measure. In their experiments to validate the mixing model we used, Lewicka-Szczebak et al. (2017) found that the model routinely underestimated gross rN₂O rates relative to measured rates in an oxic mineral soil, but performed better under anoxic conditions and in an organic soil. Therefore, an underestimation of rN₂O rates, particularly in the DS-AWD treatments, remains possible. However, considering the strong indication of N₂O reduction from other isotopocule relationships (i.e. SP and δ¹⁵N and δ¹⁸O) we believe that subsurface N₂O reduction rates were simply high in our system, regardless of water management.
In the subsurface, the contribution of denitrification/nitrifier-denitrification to \( \text{N}_2\text{O} \) concentrations was positively correlated to \( \text{N}_2\text{O}_{\text{poreair}} \) concentrations and WFPS in all treatments, indicating an increasing contribution of denitrification/nitrifier-denitrification at times of higher \( \text{N}_2\text{O} \) production in conjunction with rising soil moisture (Table 6). In the two AWD treatments, the contribution of denitrification/nitrifier-denitrification negatively correlated to \( \delta^{15}\text{N} \) signature of \( \text{N}_2\text{O}_{\text{poreair}} \) and \( \text{N}_2\text{O}_{\text{emitted}} \) (DS-AWD only). Many studies have demonstrated that high subsurface \( \text{N}_2\text{O} \) production is correlated to depleted \( \delta^{15}\text{N} \)-\( \text{N}_2\text{O} \) (Van Groenigen et al., 2005; Goldberg et al., 2008; Goldberg et al., 2010b). These results further support the conclusion that high \( \text{N}_2\text{O}_{\text{poreair}} \) and \( \text{N}_2\text{O}_{\text{emitted}} \) were produced from denitrification/nitrifier-denitrification associated with more depleted \( \delta^{15}\text{N} \)-\( \text{N}_2\text{O} \). Higher gross \( \text{rN}_2\text{O} \) (less \( \text{N}_2\text{O} \) reduction) was associated with higher \( \text{N}_2\text{O}_{\text{emitted}} \) in all treatments and higher \( \text{N}_2\text{O}_{\text{poreair}} \) (WS-AWD only), demonstrating that higher \( \text{N}_2\text{O} \) resulted not only from increased denitrification/nitrifier-denitrification but also from a decrease in \( \text{N}_2\text{O} \) reduction. Interestingly, higher \( \text{rN}_2\text{O} \) in \( \text{N}_2\text{O}_{\text{emitted}} \) of the DS-AWD was also associated with higher WFPS. Such a result can only be explained by a dependency of reduction on \( \text{N}_2\text{O} \) production. Overall, there was a negative relationship between \( \text{rN}_2\text{O} \) and \( \delta^{15}\text{N} \)-\( \text{N}_2\text{O} \), yet the relationship was not consistently strong or significant between treatments. A negative relationship supports an isotope enrichment effect with greater \( \text{N}_2\text{O} \) reduction. Considering the above, it appears that maximum \( \text{N}_2\text{O} \) production and emissions occurred during periods of increased contribution from denitrification/nitrifier-denitrification, which were accompanied by small declines in \( \text{N}_2\text{O} \) reduction. These relationships were most robust in the DS-AWD treatment. Correlations within the \( \text{N}_2\text{O}_{\text{emitted}} \) dataset were undoubtedly affected by lower data availability, particularly in the WS treatments, and should be taken with caution. Despite the high estimates of \( \text{N}_2\text{O} \) reduction for all treatments, we still observed relevant contributions from nitrification/fungal denitrification on many dates (Fig. 6). Nevertheless, the highest fluxes in the DS-AWD aligned with higher contributions from denitrification/nitrifier-denitrification, while the highest fluxes in the WS treatment had nitrification/fungal denitrification contributions of ca. 50%. In the WS treatments we again postulate that fungal denitrification rates increased because conditions were not ideal for high nitrification. Studies have shown that fungal denitrification and co-denitrification can play a significant role in soil \( \text{N}_2 \) and \( \text{N}_2\text{O} \) emissions from soil (Laughlin and Stevens, 2002; Long et al., 2013).

From our modeling results we could estimate \( \text{N}_2 \) production or emissions based on our calculated \( \text{N}_2\text{O} \) reduction rates (Fig. S13). Due to poor data availability and high variability we could neither confidently estimate \( \text{N}_2 \) production at 25 cm nor surface \( \text{N}_2 \) emissions on many dates of the WS treatments, but we have more confidence in the estimates obtained for the DS-AWD treatment. Mean daily \( \text{N}_2 \) emissions found in our study were 236 ± 53 (n=43), 194 ± 37 (n=41) and 197 ± 35 (n=31) g \( \text{N} \) ha\(^{-1}\) d\(^{-1}\) in the DS-AWD, WS-AWD and WS-FLD, respectively. To our knowledge only one other study by Yano et al. (2014) has conducted similar calculations to estimate \( \text{N}_2 \) emissions in rice systems from isotopocule signatures. The authors also found high rates of \( \text{N}_2\text{O} \) reduction, around 80 to 85%, corresponding to an \( \text{rN}_2\text{O} \) of 0.15 to 0.20 and \( \text{N}_2 \) emissions between 0.1 to 422 \( \mu \text{g} \) N m\(^{-2}\) hr\(^{-1}\) (or 0.024 to 101.4 g ha\(^{-1}\) d\(^{-1}\)). Therefore, the estimated extent of \( \text{N}_2\text{O} \) reduction was quite similar to our surface emitted reduction rates, with somewhat lower \( \text{N}_2 \) emissions corresponding to somewhat lower \( \text{N}_2\text{O} \) emissions.
Using labeled $^{15}$N urea, Lindau et al. (1990) measured $N_2$ emissions of 254 g ha$^{-1}$ d$^{-1}$, while Dong et al. (2012) observed similar rates of 194 g N$_2$N ha$^{-1}$ d$^{-1}$ for an AWD treatment. Considering that these results only account for $N_2$ derived from fertilizer, the modeled mean daily $N_2$ emissions found in our study are plausible. Differences between the treatment means were not significant for $N_2$O$_{poreair}$ or $N_2$O$_{emitted}$ ($p=0.431$ and $p=0.858$), thus do not indicate a higher potential for $N_2$ losses in the WS treatments. We must reject our hypothesis that higher NO$_3^-$ in the WS-AWD relative to the WS-FLD would drive higher denitrification and $N_2$ losses because we observed no differences in final modeled $N_2$ production and NO$_3^-$ concentrations were essentially null for both WS treatments. Our results show there is promise for estimating $N_2$ emissions from $N_2$O isotopocule signatures using simple models, but the precision of these estimates remains constrained by our ability to measure $N_2$O isotopocule signatures at low fluxes. Modeling efforts could also be refined through the implementation of a set of criteria (i.e. soil moisture status) to determine open versus closed system dynamics for a given sample.

5 Conclusions

The relatively dry conditions in the DS-AWD treatment and application of urea fertilizer led to extensive nitrification, subsequent denitrification and denitrification derived $N_2$O emissions. Even with evidence of nitrification and relatively aerobic conditions in the DS-AWD treatment, both graphical and two endmember mixing model results indicated significant $N_2$O reduction in all treatments and most convincingly in the DS-AWD treatment. Differences between depths were often more evident in $N_2$O$_{poreair}$, NO$_3^-$, NH$_4^+$ and DOC concentrations than in $N_2$O isotope signatures at the various depths, particularly for the WS treatments. In the DS-AWD treatment, isotope signatures of δ$^{18}$O-$N_2$O and SP values demonstrated notably lower values at 5 cm relative to other depths, mostly likely indicating higher $N_2$O production and less reduction in the upper layer. Overall, the highest $N_2$O production and emissions were associated with an increasing contribution from denitrification/nitrifier-denitrification accompanied by decreases in $N_2$O reduction in the AWD treatments. Our isotope data suggests that contributions from fungal denitrification to $N_2$O emissions may have increased in the WS-FLD treatment. The role of fungal denitrification in paddy rice systems should be further investigated with the use of fungal inhibitors. Surface emitted $N_2$O reduction rates were similar for all treatments, therefore our hypothesis of a greater potential for gaseous $N_2$ losses in the WS-AWD is refuted. Despite the difficulty in obtaining a full dataset for all treatments and the inherent spatiotemporal variability in the original measured fluxes, we came to good agreement with the magnitude of $N_2$ emissions reported from previous $^{15}$N labeled fertilizer studies. Thus such methods do show promise for estimating $N_2$ emissions and closing N budgets, even without the δ$^{15}$N of N substrates. Model results would likely improve with controlled incubations to determine site-specific isotope effects. Particularly in saturated or partly saturated systems, future studies should probe the disconnection between subsurface and emitted $N_2$O isotopocules by employing methods that allow for larger subsurface spatial integration, such as the installation of long horizontal gas collection tubes. It appears that to effectively manage N losses in alternative water management paddy systems inhibition of nitrification is necessary, particularly very early in the growing season when N availability exceeds crop N demand.
Dataset availability


Acknowledgements

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**Figure and table captions**

**Figure 1.** Mapping approach scheme used in the closed system modeling. Adapted from (Lewicka-Szczebak et al., 2017).

**Figure 2.** $\text{N}_2\text{O}$ surface emissions, $\log_{10}$ of dissolved and pore air $\text{N}_2\text{O}$ concentrations and major $\text{N}_2\text{O}$ driving variables ($\text{NH}_4^+$, $\text{NO}_3^-$, DOC, Eh, WFPS) throughout the field measurement period in the three water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying). The dashed vertical line indicates the date of fertilization (60 kg urea-N ha$^{-1}$). Blue shaded areas represent periods of flooding, shaded areas that last only one day indicate a ‘flush irrigation’ = flooding for < 6 hrs. The error bars represent the standard error of the mean.

**Figure 3.** Time course of $\delta^{15}\text{N}$-$\text{N}_2\text{O}$, $\delta^{18}\text{O}$-$\text{N}_2\text{O}$ and SP-$\text{N}_2\text{O}$ in $\text{N}_2\text{O}_{\text{emitted}}$ and $\text{N}_2\text{O}_{\text{poreair}}$ across the three depths and water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying). The errors bars represent the standard error of the mean.

**Figure 4.** Graphical two-end member mixing plot after Lewicka-Szczebak et al. (2017) where sample values are plotted in SP x $\delta^{18}\text{O}$-$\text{N}_2\text{O}$ space (A) and two-end mixing plot after Toyoda et al. (2011) where sample values are plotted in SP x $\delta^{15}\text{N}$-$\text{N}_2\text{O}$ space (B). In panel (a) the black dots indicate the mean literature end-member values used in our modeling scenarios and the boxes represent a range of values derived from the literature attributed to each process, see section 2.7 and Table 2. To calculate the range of $\text{N}_2\text{O}$ potentially produced by nitrification or denitrification in (B) we used the mean isotope effects, $\varepsilon^{15}\text{N}_2\text{O}_{\text{NO}_3}$ and $\varepsilon^{15}\text{N}_2\text{O}_{\text{NH}_4}$, reported in Denk et al. (2017) to represent denitrification and nitrification derived $\text{N}_2\text{O}$, respectively, and then added the minimum and maximum $\delta^{15}\text{N}$-$\text{NO}_3^-$ and $\delta^{15}\text{N}$-$\text{NH}_4^+$ values observed in each treatment (Supplementary Table 1.4). The linear relationship between each isotopocule pair is indicated in italics for all points together and for $\text{N}_2\text{O}_{\text{poreair}}$, only. The three water management treatments were: WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying.

**Figure 5.** Modeled denitrification/nitrifier-denitrification contribution and gross $\chi\text{N}_2\text{O}$ of open (grey bars), closed (blue bars) and mean (purple points and line) systems predicted by a two-endmember mixing model using $\delta^{18}\text{O}$-$\text{N}_2\text{O}$ and SP values. For open and closed system dynamics, the shaded bars represent the standard deviation range for each treatment x depth combination. The purple error bars represent the standard deviation around the mean.
Figure 6. Estimated contribution of denitrification/nitrifier-denitrification and nitrification/fungal denitrification to N\textsubscript{2}O surface emissions in the three water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying). Estimates were derived from the mean of open and closed dynamics in a two endmember mixing model using δ\textsuperscript{18}O-N\textsubscript{2}O and SP values.

Figure 7. Relationship of δ\textsuperscript{18}O-NO\textsubscript{3} to δ\textsuperscript{15}N-NO\textsubscript{3} in pore water samples of the three water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying). After Kendall and McDonnell (2012). The black arrow represents the trajectory of NO\textsubscript{3} reduction effects. The black asterisk signifies the δ\textsuperscript{18}O value atmospheric O\textsubscript{2} (25.3‰) while the dashed black line indicates the range of δ\textsuperscript{18}O in soil water. δ\textsuperscript{18}O-H\textsubscript{2}O was not directly measured in our study. We assumed a value of -8.3‰ taken from an unconfined aquifer in the region by Rapti-Caputo and Martinelli (2009). The symbol colors indicate the concentration of NO\textsubscript{3} in each sample (mg L\textsuperscript{-1}).

Table 1. Dates of management activities during the experimental period in the three water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying).

Table 2. Endmember values used for modeling of the fraction of residual N\textsubscript{2}O not reduced (gross rN\textsubscript{2}O) and the fraction of N\textsubscript{2}O + N\textsubscript{2} attributed to denitrification (gross frac\textsubscript{DEN} ) for both open and closed N\textsubscript{2}O reduction fractionation dynamics.

Table 3. Spearman correlations of N\textsubscript{2}O\textsubscript{emitted} with N\textsubscript{2}O\textsubscript{emitted} isotopocule values, N\textsubscript{2}O driving variables and N\textsubscript{2}O\textsubscript{poreair} isotopocule values measured at 5 cm in the three water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying).

Significance indicated by: **** <0.0001, *** < 0.001, **<0.01, *<0.05

Table 4. Spearman correlations between δ\textsuperscript{15}N-NO\textsubscript{3} and δ\textsuperscript{15}N-NH\textsubscript{4}\textsuperscript{+} with N\textsubscript{2}O\textsubscript{poreair} concentration, δ\textsuperscript{15}N-N\textsubscript{2}O\textsubscript{poreair}, NO\textsubscript{3} and NH\textsubscript{4}\textsuperscript{+} concentrations in the three water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying).

Table 5. ANCOVA results of modeled residual N\textsubscript{2}O not reduced (gross rN\textsubscript{2}O), fraction of total N\textsubscript{2} + N\textsubscript{2}O production coming from denitrification (gross frac\textsubscript{DEN} ) and the fraction of N\textsubscript{2}O attributed to denitrification (DenContribution) derived from N\textsubscript{2}O\textsubscript{emitted} and N\textsubscript{2}O\textsubscript{poreair}. The Y position was used a co-variates and represents the longitudinal position of each replicate within field.

Table 6. Spearman correlations between modeled rN\textsubscript{2}O-gross, frac\textsubscript{DEN} –gross and DenContribution with soil environmental variables and inorganic N substrates and δ\textsuperscript{15}N-N\textsubscript{2}O. Results are for the mean of open and closed system dynamics. Subsurface correlations were performed on data aggregated across 5 and 12.5 cm depths. Significance indicated by: **** <0.0001, *** < 0.001, **<0.01, *<0.05
Figure 1.
Figure 2.
Figure 3.

log $N_2O_{\text{pore air}}$ ($\mu g\ N\ L^{-1}$)
Figure 4.

\[ y = 0.67x - 13.5, \quad r^2 = 0.31 \] (overall)
\[ y = 0.98x - 30.0, \quad r^2 = 0.43 \] (\( N_2O_{\text{poreair}} \))

\[ y = 0.54x - 7.3, \quad r^2 = 0.11 \] (overall)
\[ y = 0.97x - 27.4, \quad r^2 = 0.27 \] (\( N_2O_{\text{poreair}} \))

\[ y = 0.31x + 5.6, \quad r^2 = 0.04 \] (overall)
\[ y = 0.98x - 26.3, \quad r^2 = 0.29 \] (\( N_2O_{\text{poreair}} \))

\[ y = 0.62x + 20.7, \quad r^2 = 0.36 \] (overall)
\[ y = 0.83x + 19.5, \quad r^2 = 0.50 \] (\( N_2O_{\text{poreair}} \))

\[ y = 0.06x + 17.1, \quad r^2 = 0.003 \] (overall) NS
\[ y = 0.10x + 16.0, \quad r^2 = 0.01 \] (\( N_2O_{\text{poreair}} \)) NS

\[ y = 0.07x + 19.6, \quad r^2 = 0.004 \] (overall) NS
\[ y = 0.49x + 20.4, \quad r^2 = 0.14 \] (\( N_2O_{\text{poreair}} \))
Figure 5.
Figure 6.
Figure 7.
Table 1. Dates of management activities during the experimental period in the three water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying).

<table>
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<tr>
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<td>31-May; 6-Jun; 10-Jun</td>
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<td>21-Jun (60 kg ha⁻¹)</td>
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Discussion started: 11 July 2018
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Table 2. Endmember values used for modeling of the fraction of residual N$_2$O not reduced (gross rN$_2$O) and the fraction of N$_2$O + N$_2$ attributed to denitrification (gross frac$_{DEN}$) for both open and closed N$_2$O reduction fractionation dynamics.

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<tr>
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<th>references</th>
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<td>-15</td>
<td>-5</td>
<td>Lewicka-Szczebak et al. (2017)</td>
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*Lewicka-Szczebak et al. (2017) originally report $\delta^{18}$O-N$_2$O(N$_2$O/H$_2$O). Thus, to calculate a pure $\delta^{18}$O-N$_2$O, we added the $\delta^{18}$O-H$_2$O value used in our study, -8.3%.

Table 3. Spearman correlations of N$_2$O$_{emitted}$ with N$_2$O$_{emitted}$ isotopocule values, N$_2$O driving variables and N$_2$O$_{poreair}$ isotopocule values measured at 5 cm in the three water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying). Significance indicated by: **** <0.0001, *** < 0.001, **<0.01, *<0.05

<table>
<thead>
<tr>
<th>N$<em>2$O$</em>{emitted}$</th>
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<th>$\delta^{18}$O-N$<em>2$O$</em>{emitted}$</th>
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</tr>
<tr>
<td>NO$<em>3$-N$</em>{porewater}$, 5cm</td>
<td>-0.21</td>
<td>0.1</td>
<td>0.52***</td>
</tr>
<tr>
<td>NH$<em>4$-N$</em>{porewater}$, 5cm</td>
<td>-0.29*</td>
<td>-0.32*</td>
<td>-0.31</td>
</tr>
<tr>
<td>$\delta^{15}$N-N$<em>2$O$</em>{poreair}$, 5cm</td>
<td>0.24</td>
<td>0.09</td>
<td>-0.51****</td>
</tr>
<tr>
<td>$\delta^{18}$O-N$<em>2$O$</em>{poreair}$, 5cm</td>
<td>-0.07</td>
<td>0.07</td>
<td>-0.39**</td>
</tr>
<tr>
<td>$\delta$SP-N$<em>2$O$</em>{poreair}$, 5cm</td>
<td>-0.27</td>
<td>-0.1</td>
<td>-0.55****</td>
</tr>
</tbody>
</table>
Table 4. Spearman correlations between δ^{15}N-NO_3^- and δ^{15}N-NH_4^+ with N_2O_poreair concentration, δ^{15}N-N_2O_poreair, NO_3^- and NH_4^+ concentrations in the three water management treatments (WS-FLD = water-seeding + conventional flooding; WS-AWD = water-seeding + alternate wetting and drying; DS-AWD = direct dry seeding + alternate wetting and drying).

<table>
<thead>
<tr>
<th></th>
<th>DS-AWD</th>
<th>WS-AWD</th>
<th>WS-FLD</th>
<th>DS-AWD</th>
<th>WS-AWD</th>
<th>WS-FLD</th>
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</thead>
<tbody>
<tr>
<td>δ^{15}N-NO_3^-</td>
<td>-0.54*</td>
<td>-0.03</td>
<td>-0.05</td>
<td>-0.54*</td>
<td>-0.03</td>
<td>-0.05</td>
</tr>
<tr>
<td>δ^{15}N-NH_4^+</td>
<td>-0.54*</td>
<td>-0.03</td>
<td>-0.05</td>
<td>-0.54*</td>
<td>-0.03</td>
<td>-0.05</td>
</tr>
<tr>
<td>N_2O_poreair</td>
<td>0.34**</td>
<td>0.07</td>
<td>0.38**</td>
<td>-0.72***</td>
<td>0.04</td>
<td>0.22*</td>
</tr>
<tr>
<td>δ^{15}N-N_2O_poreair</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.14</td>
<td>0.46*</td>
<td>-0.03</td>
<td>0.14</td>
</tr>
<tr>
<td>NO_3^-</td>
<td>-0.66****</td>
<td>-0.01</td>
<td>-0.28</td>
<td>-0.41</td>
<td>0.11</td>
<td>0.27*</td>
</tr>
<tr>
<td>NH_4^+</td>
<td>0.01</td>
<td>0.13</td>
<td>-0.06</td>
<td>-0.54*</td>
<td>-0.23*</td>
<td>-0.12</td>
</tr>
</tbody>
</table>

Table 5. ANCOVA results of modeled residual N_2O not reduced (gross rN_2O), fraction of total N_2 + N_2O production coming from denitrification (gross frac_{DEN}) and the fraction of N_2O attributed to denitrification (DenContribution) derived from N_2O_emitted and N_2O_poreair. The Y position was used a co-variate and represents the longitudinal position of each replicate within field.

<table>
<thead>
<tr>
<th></th>
<th>NumDF</th>
<th>N_2O_poreair rN_2O-gross</th>
<th>N_2O_poreair frac_{DEN} - gross</th>
<th>DenContribution (N_2O_poreair)</th>
<th>NumDF</th>
<th>N_2O_emitted rN_2O-gross</th>
<th>N_2O_emitted frac_{DEN} - gross</th>
<th>DenContribution (N_2O_emitted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>treatment</td>
<td>2</td>
<td>0.004</td>
<td>&lt;0.001</td>
<td>0.188</td>
<td>2</td>
<td>0.146</td>
<td>0.931</td>
<td>0.016</td>
</tr>
<tr>
<td>day</td>
<td>14</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>16</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>depth</td>
<td>1</td>
<td>0.019</td>
<td>0.007</td>
<td>0.008</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Y position</td>
<td>1</td>
<td>0.844</td>
<td>0.016</td>
<td>0.375</td>
<td>1</td>
<td>0.451</td>
<td>0.373</td>
<td>0.818</td>
</tr>
<tr>
<td>trmt:day</td>
<td>28</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>19</td>
<td>0.009</td>
<td>0.024</td>
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<tr>
<td>trmt:depth</td>
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<tr>
<td>day:depth</td>
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<td>&lt;0.001</td>
<td>0.002</td>
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<td>trmt:day:depth</td>
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<td>0.022</td>
<td>0.047</td>
<td>0.189</td>
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</tbody>
</table>
Table 6. Spearman correlations between modeled $r_{N_2O}$-gross, $\text{frac}_{DEN}$--gross and $DenContribution$ with soil environmental variables and inorganic N substrates and $\delta^{15}N$-$N_2O$. Results are for the mean of open and closed system dynamics. Subsurface correlations were performed on data aggregated across 5 and 12.5 cm depths. Significance indicated by: **** <0.0001, *** < 0.001, **<0.01, *<0.05

<table>
<thead>
<tr>
<th></th>
<th>$\text{frac}_{DEN}$-gross</th>
<th>$r_{N_2O}$-gross</th>
<th>$DenContribution$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[N_2O_{\text{poreair}}]$</td>
<td>0.34****</td>
<td>0.2</td>
<td>0.31*</td>
</tr>
<tr>
<td>WFPS</td>
<td>0.21*</td>
<td>0.21*</td>
<td>0.39**</td>
</tr>
<tr>
<td>Eh</td>
<td>-0.04</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>0.16</td>
<td>0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>-0.22</td>
<td>-0.06</td>
<td>-0.19</td>
</tr>
<tr>
<td>$\delta^{15}N$-$N_2O_{\text{poreair}}$</td>
<td>-0.35****</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[N_2O_{\text{emitted}}]$</td>
<td>-0.21</td>
<td>-0.73****</td>
<td>-0.40*</td>
</tr>
<tr>
<td>WFPS</td>
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<td>0.18</td>
</tr>
<tr>
<td>Eh</td>
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<td>0.08</td>
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<tr>
<td>NO$_3^-$</td>
<td>-0.44**</td>
<td>-0.17</td>
<td>-0.28</td>
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<tr>
<td>NH$_4^+$</td>
<td>0.39*</td>
<td>0.52**</td>
<td>0.59**</td>
</tr>
<tr>
<td>$\delta^{15}N$-$N_2O_{\text{emitted}}$</td>
<td>0.60****</td>
<td>0.29</td>
<td>0.36</td>
</tr>
</tbody>
</table>