Sources of nitrous oxide from intensively managed pasture soils: the hole in the pipe

To cite this article: Johannes Friedl et al 2021 Environ. Res. Lett. 16 065004

View the article online for updates and enhancements.
Sources of nitrous oxide from intensively managed pasture soils: the hole in the pipe

Johannes Friedl1, Clemens Scheer1,2, Daniele De Rosa1, Christoph Müller3,4, Peter R Grace1 and David W Rowlings1

1 Centre for Agriculture and the Bioeconomy, Queensland University of Technology, Brisbane, QLD 4000, Australia
2 Institute for Meteorology and Climate Research (IMK-IFU) Karlsruhe Institute of Technology (KIT), Garmisch-Partenkirchen, Germany
3 Institute of Plant Ecology (IFZ), Justus-Liebig University, Giessen, Germany
4 School of Biology and Environmental Science, University College Dublin, Belfield, Dublin, Ireland

* Authors to whom any correspondence should be addressed.
E-mail: johannes.friedl@qut.edu.au and clemens.Scheer@kit.edu

Keywords: nitrous oxide, greenhouse gas emissions, climate change, denitrification, nitrification, pastures

Abstract
Rainfall and irrigation trigger large pulses of the powerful greenhouse gas N\textsubscript{2}O from intensively managed pastures, produced via multiple, simultaneously occurring pathways. These N\textsubscript{2}O pulses can account for a large fraction of total N\textsubscript{2}O losses, demonstrating the importance to determine magnitude and source partitioning of N\textsubscript{2}O under these conditions. This study investigated the response of different pathways of N\textsubscript{2}O production to wetting across three different textured pasture soils. Soil microcosms were fertilised with an ammonium nitrate (NH\textsubscript{4}NO\textsubscript{3}) solution which was either single or double \textsuperscript{15}N labelled, wetted to four different water-filled pore space (WFPS) levels, and incubated over two days. The use of a \textsuperscript{15}N pool mixing model together with soil N gross transformations enabled the attribution of N\textsubscript{2}O to specific pathways, and to express N\textsubscript{2}O emissions as a fraction of the underlying N transformation. Denitrification and nitrification mediated pathways contributed to the production of N\textsubscript{2}O in all soils, regardless of WFPS. Denitrification was the main pathway of N\textsubscript{2}O production accounting for >50% of cumulative N\textsubscript{2}O emissions even at low WFPS. The contribution of autotrophic nitrification to N\textsubscript{2}O emissions decreased with the amount of wetting, while the contribution of heterotrophic nitrification remained stable or increased. Following the hole-in-the-pipe model, 0.1%–4% of nitrified N was lost as N\textsubscript{2}O, increasing exponentially with WFPS, while the percentage of denitrified N emitted as N\textsubscript{2}O decreased, providing critical information for the representation of N\textsubscript{2}O/WFPS relationships in simulation models. Our findings demonstrate that the wetting of pasture soils promotes N\textsubscript{2}O production via denitrification and via the oxidation of organic N substrates driven by high carbon and N availability upon wetting. The large contribution of heterotrophic nitrification to N\textsubscript{2}O emissions should be considered when developing N\textsubscript{2}O abatement strategies, seeking to reduce N\textsubscript{2}O emissions in response to rainfall and irrigation from intensively managed pastures.

1. Introduction
Pasture soils are a major source of nitrous oxide (N\textsubscript{2}O), a powerful greenhouse gas with a global warming potential 298 times higher than that of carbon dioxide (CO\textsubscript{2}) (Myhre et al 2013) and the single most depleting substance of stratospheric ozone in this century (Ravishankara et al 2009). High carbon (C) (Li et al 2005, Morley and Baggs 2010) and nitrogen (N) substrate availability (Kim et al 2013, Van Lent et al 2015) promote emissions of N\textsubscript{2}O from pasture soils. In intensively managed pasture systems such as dairy pastures, N substrate availability for N\textsubscript{2}O production is further increased by N
inputs in the form of urine and dung (Clough et al. 2020) and N fertiliser (Stott and Gourley 2016). Large pulses of N$_2$O from pasture soils are triggered by rainfall (Rowlings et al. 2015) and irrigation (Mumford et al. 2019). Rainfall variability in subtropical regions is high (Murphy and Ribbe 2004, Rowlings et al. 2015), and over the coming decades, pastures in these regions will be subjected to further increasing drying and wetting cycles due to the predicted changes in global climate. The cascade of N transformations triggered by the wetting of dry soil (Borken and Matzner 2009) produces N$_2$O via a multitude of different production pathways, fuelled by the sudden increase in soil water content, microbial activity and N substrate availability. Predictions on the processes contributing and dominating N$_2$O production under these conditions are highly uncertain despite an increasingly well-defined mechanistic understanding of N$_2$O production (Bakken and Frostegård 2017, Yoon et al. 2019), and the representation of N$_2$O pulses triggered by wetting in simulation models remains challenging (Bessou et al. 2010, Fuchs et al. 2020). These challenges reflect the lack of systematic research including different N$_2$O production pathways, the reduction of N$_2$O to dinitrogen (N$_2$), and underlying gross N transformations in response to wetting pulses.

In the conceptual hole-in-the-pipe (HIP) model (Firestone and Davidson 1989, Zhang et al. 2015), N$_2$O emissions are depicted as the fraction $R_{N2O}$ of the underlying N transformation. This conceptual framework is widely used in simulation models such as DACE (Nepálová et al. 2015), DNDC (Li et al. 2006), LDPNDC (Haas et al. 2013) and NOE (Hénault et al. 2005). The size of the pipe represents the rate of the N transformation, while the hole is the respective fraction ($R$) that is emitted as N$_2$O, defined by physical and chemical factors such as soil moisture, temperature, and soil pH. The main pools considered are the ammonium (NH$_4$$^+$) and nitrate (NO$_3^-$) pool, and the model attributes N$_2$O formation to these pools, and the respective N transformations, i.e. nitrification and denitrification. Following the HIP model, N$_2$O production pathways via nitrification include the chemical decomposition of hydroxylamine (Heil et al. 2015) and the reduction of nitrite (NO$_2^-$) by autotrophic nitrifiers, i.e. nitrifier-denitrification, (Wragge-Mönning et al. 2018), while NO$_3^-$ is regarded as the sole source pool of N$_2$O formation via denitrification. Analogue to the HIP model, stable isotope tracing methods, based on the $^{15}$N labelling of the NH$_4$$^+$ and/or NO$_3^-$ pool have been widely used to quantify N gross transformation rates (Kirkham and Bartholomew 1954, Müller et al. 2004) and associated N$_2$O emissions (Müller et al. 2014) based on a two-source model (Stevens et al. 1997). In contrast to the use of inhibitors (Berg et al. 1982, Hynes and Knowles 1982), or analysis of the isotopic composition of N$_2$O without the addition of stable isotopes (Decock and Six 2013, Yu et al. 2020), this approach does not account for specific microbial processes but aligns with the representation of N$_2$O production in biochemical models, recommending its use to establish the contribution of specific N transformation to N$_2$O production.

Two pathways of N$_2$O production linked to organic N pool have recently gained more attention due to the inclusion of the organic N pool in $^{15}$N$_2$O tracing models: heterotrophic nitrification of organic N (Zhang et al. 2015) and co-denitrification (Clough et al. 2017, Rex et al. 2019). The formation of N$_2$O via heterotrophic nitrification is thought to occur via the oxidation of organic N to NO$_2^-$ and its subsequent reduction to N$_2$O (Braker and Conrad 2011). Although heterotrophic nitrifiers can use a wide range of substrates including NH$_4$$^+$ (Stein 2011), we refer to this pathway in the context of $^{15}$N source partitioning as N$_2$O production from organic N compounds only. Besides classic denitrification, co-denitrification can also contribute to N$_2$O production, forming hybrid N$_2$O by combining an inorganic N compound such as NO$_2^-$ with a co-metabolised organic N-substrate (Spott et al. 2011).

Source partitioning of N$_2$O from temperate pastures has demonstrated the significance of both heterotrophic nitrification (Müller et al. 2014, Jansen-Willem et al. 2016, Moser et al. 2018) and co-denitrification (Selbie et al. 2015, Rex et al. 2019) for N$_2$O production, yet their significance in response to different degrees of wetting remains largely unknown. More importantly, assessments of N$_2$O production pathways in response to soil water content (Bateman and Bags 2005, Mathieu et al. 2006, Loick et al. 2021) mostly lack data on N$_2$O reduction to N$_2$. This hinders the quantification of overall denitrification, and thus the assessment of denitrification as source and sink of N$_2$O upon wetting.

The aim of this study was therefore to establish the response and significance of nitrification and denitrification mediated pathways of N$_2$O production across three different textured pasture soils exposed to different degrees of wetting, and to quantify what fraction of the underlying N transformation is emitted as N$_2$O. We combined $^{15}$N$_2$O analysis and a $^{15}$N$_2$O pool mixing model with soil N transformation and N$_2$O data presented in Friedel et al. (2018), allowing the calculation of the contribution of nitrification and denitrification (N$_2$ + N$_2$O) emitted as N$_2$O following the HIP model, and to derive the response curves of these fractions across different soil water contents. As such, this study addresses a major uncertainty in biochemical models simulating the N cycle: the fraction of N$_2$O emitted from denitrification ($RN_2O_4$), the magnitude of overall denitrification (Del Grosso et al. 2020), and the fraction of N$_2$O...
emitted via nitrification mediated pathways (RN₂O₅) (Chen et al. 2008).

2. Material and methods

Soil samples (0–10 cm) were collected from three intensively managed dairy pastures in subtropical Australia. Emissions of N₂O from these pasture sites were previously quantified in both laboratory-based (Friedl et al. 2016, 2020) and field-based experiments (Friedl et al. 2017, Mumford et al. 2019, De Rosa et al. 2020). The site location and characteristics, including physical and chemical soil properties, are shown in Table 1. The soils were classified as pellic Vertisol, ferric Acrisol and mollic Fluvisol, respectively (IUSS Working Group 2015), and are henceforth referred to as clay, loam and sandy clay loam (sandy CL), according to their texture from 0 to 10 cm. The organic C content of the soils ranges from 4.1% to 4.9%, following the order sandy CL < clay < loam (Table 1). The soil pH measured in water (1:5, v:w) is 6.3, 6.1 and 5.9 for the clay, the loam, and the sandy CL, respectively.

Table 1. Selected soil characteristics (0–10 cm) for three intensively managed pasture sites under dairy production in subtropical Australia.

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Clay</th>
<th>Loam</th>
<th>Sandy clay loam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>Casino</td>
<td>Gympie</td>
<td>Kerry</td>
</tr>
<tr>
<td>Longitude</td>
<td>152.874</td>
<td>152.74</td>
<td>153.031</td>
</tr>
<tr>
<td>Mean annual rainfall</td>
<td>1107 mm</td>
<td>1127 mm</td>
<td>906.7 mm</td>
</tr>
<tr>
<td>Soil type (ASC)</td>
<td>Vertisol</td>
<td>Dermosol</td>
<td>Tenosol</td>
</tr>
<tr>
<td>Soil type (FAO)</td>
<td>Pellic Vertisol</td>
<td>Ferric Acrisol</td>
<td>Mollic Fluvisol</td>
</tr>
<tr>
<td>Texture (USDA)</td>
<td>Clay</td>
<td>Loam</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>pH (water, 1:5)</td>
<td>6.3</td>
<td>6.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Organic Carbon (%)</td>
<td>4.2</td>
<td>4.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Total Nitrogen (%)</td>
<td>0.36</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>11.4</td>
<td>9.8</td>
<td>10.4</td>
</tr>
</tbody>
</table>

2.1. ¹⁵N tracing experiment

The experiment was set up in a full factorial design with four different water-filled pore space (WFPS) levels across three different textured pasture soils and four replicates using a triple N labelling approach combined with a ¹⁵N tracing model (Friedl et al. 2018). In the study presented here, we used additional ¹⁵N₂O gas analysis together with gross N transformation data presented in Friedl et al. (2018) to attribute N₂O losses to specific N₂O production pathways.

The experimental setup is described in Friedl et al. (2018). Briefly, soil collected from the three pasture sites was partially air-dried (10% gravimetric water content) and sieved to 4 mm. Soil microcosms were established in 50 ml centrifuge tubes using the equivalent of 8 g oven-dry soil. One milliliter of NH₄NO₃ solution containing the equivalent of 35 µg N g⁻¹ soil was applied to each microcosm, either single (NH₄¹⁵NO₃) labelled (a) or double (¹⁵NH₄¹⁵NO₃) labelled (b) at 10 atom %. Soil microcosms were wetted to 40, 60, 80 and 95% WFPS and the soil was compacted to a volume of 8 ml using a plunger, resulting in an adjusted bulk density of 1 g cm⁻³. Homogenous labelling was ensured by applying water and fertiliser solution dropwise on two layers of 4 g of soil. Centrifuge tubes were then closed with suba-seals (Sigma Aldrich) and kept closed in an incubator at a constant temperature of 25 °C between gas sampling events.

2.2. Gas samples

Ambient background air samples (n = 4) were taken each day before closing the centrifuge tubes. The headspace atmosphere of treatment (a) and (b) was sampled 24 and 48 h after closure using a gastight syringe. The suba-seals were removed after the first sampling event for 10 min to allow gas exchange with the headspace atmosphere. Gas samples were transferred into pre-evacuated 12 ml Exetainer tubes with a double wadded Teflon/silicon septa cap (Labco Ltd, Buckinghamshire, UK) and stored until N₂O and CO₂ analysis by gas chromatography (Shimadzu GC-14). Gas samples were analysed for isotopologues of N₂O (¹⁴N¹⁴N, ¹⁴N¹⁵N and ¹⁵N¹⁵N, and ¹⁴N¹⁴N¹⁵O) using an automated isotope ratio mass spectrometer (IRMS) (Sercon Limited, 20–20, UK). Due to instrument malfunction, headspace samples from the sandy clay loam from treatment (a) and (b) at 95% WFPS were lost and could not be analysed for ¹⁵N₂O.

2.3. Gross N transformations

Data on gross N transformations were derived from Friedl et al. (2018). In brief: soil mineral N was extracted using 40 ml of 2 M KCl 30 min (t = 0) and 48 h (t = 2 d) after fertiliser application. Four additional soil microcosms per ¹⁵N treatment were used for t = 0 extractions, and all soil microcosms (treatment a and b) were extracted at t = 2 d. The ¹⁵N enrichment of the soil mineral N pool was determined by the diffusion method (Stark and Hart 1996). Gross N transformations were quantified using the ¹⁵N tracing tool Ntrace (Müller et al. 2007; Zaman et al. 2021), using a Markov Chain Monte Carlo method to optimise the fit of the data to the conceptual N cycling model.
shown in figure S1 (available online at stacks.iop.org/ERL/16/065004/mmedia). This model comprises five N pools linked by ten different N transformations. Autotrophic nitrification is defined as the oxidation of NH$_4^+$ to NO$_3^-$ ($O_{NH4}$) and heterotrophic nitrification as the oxidation of recalcitrant organic N to NO$_3^-$ ($O_{Nrec}$), given in µg N g$^{-1}$ soil.

2.4. Fluxes of N$_2$O and CO$_2$ and N$_2$O source partitioning

Fluxes of N$_2$O and CO$_2$ were determined using the closed chamber method and calculated from the slope of the assumed linear increase in gas concentration during the closure period, corrected for temperature and air pressure (Scheer et al. 2014). The $^{15}$N fraction of N$_2$O was then used to attribute N$_2$O production to source pools of a specific $^{15}$N enrichment using a pool mixing model. The model assumes uniform $^{15}$N labelling of the soil mineral N pools and negligible isotopic discrimination for all N$_2$O production pathways.

IRMS analysis of the headspace samples of treatment (a) and (b) at $t = 24$ h and $t = 48$ h provided the ion currents (I) at m/z 44,45 and 46 enabling the molecular ratios $^{45}R$ ($^{15}O/^{16}O$) and $^{46}R$ ($^{16}O/^{16}O$) to be calculated. Possible N$_2$O species include also $^{47}R$ and $^{48}R$ and were calculated as

$$^{47}R = (15R)^2 \times 17R + 2 + 15R \times 18R$$

where $^{17}R$ ($^{17}O/^{16}O$) = 0.00037795 and $^{18}R$ ($^{18}O/^{16}O$) = 0.002079 as $^{17}R$ and $^{18}R$ are assumed to be at natural abundance.

The $^{15}$N fraction of N$_2$O is then calculated as

$$a_{N_2O} = \frac{45R + 2 \times 46R - 17R - 2 \times 18R}{(2 + 2 \times 45R + 2 \times 46R)}.$$  

Emissions of N$_2$O from treatment $a$ and $b$ were attributed to four different source pathways including three different N pools: denitrification derived from the NO$_3^-$ pool at enrichment $a_d$, autotrophic nitrification ($O_{NH4}$), the oxidation of the NH$_4^+$ pool at enrichment $a_a$, heterotrophic nitrification ($O_{Nrec}$), defined as the oxidation of organic N at natural abundance $a_o$, and hybrid formation of N$_2$O attributed to co-denitrification. Hybrid N$_2$O is formed by one N atom from the NO$_3^-$ pool and one from the organic N pool. The $^{15}$N fractional enrichment of N$_2$O is given by equation (4) as

$$a_{N_2O} = d \times a_d + n \times a_a + h \times a_o + c d \times (0.5 \times (a_d + a_o))$$

where $a_{N_2O}$ is the $^{15}$N enrichment of the respective headspace sample, and d, n, h, and cd are the fraction of N$_2$O emitted via denitrification, autotrophic nitrification, heterotrophic nitrification and co-denitrification, respectively. Daily values for $a_d$ and $a_a$ were calculated as daily average, assuming a linear increase/decrease of the respective $^{15}$N enrichment over the time of the experiment (from $t = 0$ to $t = 2$ d). For each headspace sample, d, n, and cd were quantified using the Excel SOLVER (Microsoft Excel 2016) by minimising the absolute difference between measured and calculated $^{15}$N enrichment of N$_2$O using all possible combinations between the different replicates of treatment (a) and (b) ($n = 16$). The fraction $h$ was calculated as

$$h = 1 - d - n - cd$$

where all the N$_2$O assumed to come from one of the four N$_2$O production pathways. Average contributions were calculated using the values for $d$, $n$, $h$, and cd when the SOLVER solution satisfied all the constraints. The SOLVER function did not find a feasible solution for 18% of the combinations. Multiplying the N$_2$O flux with $d$, $n$, $h$, and cd gave the amount of N$_2$O emitted via denitrification (N$_2$O$_d$), autotrophic nitrification (N$_2$O$_a$), heterotrophic nitrification (N$_2$O$_h$) and co-denitrification (N$_2$O$_cd$), respectively. Emissions of N$_2$O produced by all nitrification mediated pathways (N$_2$O$_h$) were calculated as the sum of N$_2$O$_d$, N$_2$O$_a$, and N$_2$O$_h$. Following the hole in the pipe model, the fraction of N$_2$O lost via denitrification was expressed as

$$RN_{2O_d} = \frac{N_{2O_d}}{Den}$$

where Den was overall denitrification, calculated using the product ratio of denitrification N$_2$O/(N$_2$ + N$_2$O) obtained in Friedl et al (2018) (treatment c) and the N$_2$O$_d$ from treatment (a) and (b). The RN$_2$O$_a$ factors for autotrophic nitrification ($O_{NH4}$) and heterotrophic nitrification ($O_{Nrec}$) were calculated accordingly using equations (7) and (8):

$$RN_{2O_h} = \frac{N_{2O_h}}{O_{Nrec}}$$

The factor RN$_2$O$_a$ for total nitrification was calculated as

$$RN_{2O_h} = \frac{N_{2O_h}}{O_{Nrec} + O_{NH4}}.$$

2.5. Statistical analysis

Statistical analyses were conducted with SPSS 22.0 (SPSS Inc. 2013). The effects of soil texture and WFPS on cumulative emissions of N$_2$O$_d$, N$_2$O$_a$, N$_2$O$_h$, and CO$_2$ were examined by analysis of variance (ANOVA) ($P < 0.05$). Normal distribution
of the data was assessed by the Shapiro–Wilk test for normality. Tukey’s honest significant difference (HSD) test was used to determine differences between pasture soils within a WFPS treatment, and within a pasture soil across different WFPS. The response of cumulative \( N_2O_d \), \( N_2O_a \), and \( N_2O_h \) emissions to WFPS was quantified with generalised additive models (GAM) utilising the R package mgcv (Wood 2015). GAMs are semi-parametric models and can test and quantify the non-linear relationship between response and explanatory variables. The Akaike Information Criterion (AIC) and the deviance explained aided for model selection (Akaike 1974). Due to analytical problems, \( N_2O \) source partitioning for the sandy clay loam at 95% WFPS is missing, and therefore not considered when evaluating differences between soils or the response to different WFPS. The relationship between N transformations and \( RN_2O \) values vs. WFPS was evaluated by regression analysis using SPSS 22.0 and SigmaPlot Version 13.0. Results of the regression analysis, including the best-fit model and parameters for figures 2 and 3 are given in table S1. Values in the text, tables and figures represent means ± standard error of the mean.

3. Results

3.1. Cumulative \( N_2O \) emissions
Cumulative \( N_2O \) emissions ranged from 0.04 to >7 \( \mu g \ N_2O-N \ g^{-1} \) soil and differed between soil type, following the order clay > loam > sandy CL, regardless of WFPS (figure 1, table 2). Emissions of \( N_2O \) increased exponentially with increasing WFPS, except for the loam, where \( N_2O \) emissions increased exponentially up to 80% WFPS and decreased at WFPS > 80%.

3.2. Contribution of different \( N_2O \) production pathways to cumulative \( N_2O \) emissions
Denitrification, autotrophic nitrification, and heterotrophic nitrification contributed to the production of \( N_2O \) in all soils, regardless of WFPS (table 2, figure 1). Denitrification was the main \( N_2O \) production pathway in the clay and the loam at all WFPS levels and at 40 and 80% WFPS in the sandy CL, accounting for 40%–82% of cumulative \( N_2O \) emissions (table 2). Emissions of \( N_2O_d \) were highest in the clay, followed by the loam and the sandy CL, and increased exponentially with increasing WFPS, except for the loam, where \( N_2O_d \) emissions decreased at WFPS > 80% following an exponential increase at WFPS levels <80%. The magnitude of \( N_2O_d \) emissions was smaller than the combined error of GC analysis. \( ^{15}N_2O \) analysis and the quantification of the fraction of \(^{15}N \) in the soil \( NH_4^+ \) and \( NO_3^- \) pool, and therefore assumed to be below the method detection limit.

Emissions of \( N_2O_a \) increased with soil WFPS in the clay and the loam, with highest \( N_2O_a \) emissions observed at 80 and 95% WFPS in the clay, and at 95% in the loam. Emissions of \( N_2O_a \) from the sandy CL were negatively correlated to increasing soil WFPS and accounted for less than 23% of cumulative \( N_2O \) emissions. Across soils, \( N_2O_a \) emissions increased with increasing WFPS reaching a plateau at WFPS > 80%. Heterotrophic nitrification exceeded autotrophic nitrification as an \( N_2O \) production pathway in the clay and the sandy CL regardless of WFPS, but its contribution to \( N_2O \) emissions remained below the one of autotrophic nitrification in the clay. Emissions of \( N_2O \) from all nitrification mediated pathways, calculated as the sum \( N_2O_a \) and \( N_2O_h \) increased with soil WFPS, with highest \( N_2O \) emissions observed > 80% WFPS.

3.3. The fraction of different N transformations emitted as \( N_2O \)
Denitrification and total nitrification (the sum of autotrophic and heterotrophic nitrification) and the fraction of nitrified and denitrified N emitted as \( N_2O \) (\( RN_2O \)) are shown in figure 2. Denitrification (\( N_2 + N_2O_d \)) increased exponentially with increasing WFPS (table S2), with no difference in magnitude between soils. The fraction of denitrified N emitted as \( N_2O \) (\( RN_2O \)) followed a linear decrease with increasing WFPS in the loam and the sandy CL, and in the clay after increasing from 40% to 60% WFPS.

The response of total nitrification to WFPS differed between soils (figure 2), showing a slight increase from 40% to 80% WFPS in the clay and the sandy CL, while nitrification rates peaked at 80% WFPS in the loam at >40 \( \mu g \ NO_3^-N \ g^{-1} \) d\(^{-1} \) soil and decreased thereafter. Across all soils, \( RN_2O \) ranged from 0.002 to 0.042 and increased exponentially with increasing WFPS.

The response of autotrophic and heterotrophic nitrification to WFPS and respective \( RN_2O \) values is shown in figure 3. In the clay and the sandy CL, autotrophic nitrification peaked between 60 and 80% WFPS at >20 \( \mu g \ NO_3^-N \ g^{-1} \) d\(^{-1} \) soil day\(^{-1} \), and decreased to less than 2 \( \mu g \ NO_3^-N \ g^{-1} \) d\(^{-1} \) at 95% WFPS. In the loam, autotrophic nitrification remained <1.5 \( \mu g \ NO_3^-N \ g^{-1} \) d\(^{-1} \) across all WFPS levels. The fraction \( RN_2O \) from the clay and the loam responded with an exponential increase to increasing WFPS, with values up to 1 and 0.4 observed at 95% WFPS in the clay and the loam, respectively. The fraction \( RN_2O \) from the sandy CL decreased from 0.013 to <0.001 from 40% to 80% WFPS, following an exponential decay curve.

Heterotrophic nitrification was the dominant nitrification pathway in the loam at all WFPS levels and at 95% WFPS in the clay and the loam (figure 3). Rates of heterotrophic nitrification followed a quadratic function in the clay and the sandy clay loam, with the lowest rates observed at 60% WFPS, and a subsequent increase up to 8 and 25 \( \mu g \ NO_3^-N \ g^{-1} \) soil day\(^{-1} \) at 95% WFPS, respectively. Heterotrophic nitrification peaked between 60 and 80% WFPS in
Figure 1. Cumulative N₂O emissions and the contribution of heterotrophic nitrification, autotrophic nitrification, co-denitrification and denitrification to N₂O emissions in response to increasing soil water filled pore space across three different textured pasture soils.

the loam at >than 40 µg NO₃⁻N g⁻¹ soil day⁻¹ and decreased thereafter. Highest values for RN₂Oₗ were observed from the sandy CL peaking at 0.95 at 60% WFPS, dropping to 0.31 at 80% WFPS. In the clay and the loam, RN₂Oₗ remained below 0.04, peaking at 80 and 95% WFPS, respectively.

3.4. Labile C availability and cumulative CO₂ emissions
Labile C availability prior incubation was derived from Friedl et al (2018). Labile C measured as permanganate-oxidisable C (Weil et al 2003) was highest in the loam (1196 ± 14.9 µgC g⁻¹ soil),
4. Discussion

Wetting events trigger pulses of N₂O emissions from soils, accounting for a large proportion of overall N₂O emissions. The relative change in soil water content together with the antecedent soil moisture, rather than absolute amounts of water in soil define the magnitude of these N₂O pulses (Bergstromm et al 2011, Harris et al 2021). The short-term response of different N₂O production pathways to wetting of pasture soils provides therefore critical information to constrain magnitude and source partitioning of N₂O pulses. This study demonstrates the simultaneous occurrence of N₂O emissions via denitrification and nitrification mediated pathways across three different pasture soils regardless of the amount of wetting. Partitioning of N₂O emission shows the large contribution of heterotrophic nitrification to N₂O production, highlighting the oxidation of organic N as a major source of N₂O from pasture soils. Following the HIP model, the fraction of total nitrification lost as N₂O (RN₂O₅) ranged from 0.001 to 0.04 and increased exponentially with soil WFPS, while the respective fraction of denitrification (RN₂O₃) decreased. Based on these findings, we postulate that (a) in pasture soils with high organic C and N content, the cascade of physical, chemical and biological processes triggered by wetting promotes N₂O production via denitrification and via the oxidation of organic N substrates and (b) that the exponential increase of the hole in the pipe, i.e. the amount of N₂O lost from nitrification mediated pathways is driven by the denitrification of nitrified N.

4.1. Production of N₂O from denitrification and co-denitrification in response to wetting

The use of the 15N pool mixing model showed that denitrification dominated N₂O production in all soils (figure 1), accounting for 40%–80% of cumulative N₂O losses. Under oxic conditions (≤60% WFPS), production of N₂O is assumed to occur.
Figure 2. Rates of denitrification ($N_2 + N_2O$) and total nitrification and the respective fraction of these nitrogen transformations emitted as $N_2O$ ($RN_2O_2$ and $RN_2O_3$, respectively). Note the difference in scale for denitrification and nitrification, with maximum values of 7 and 52 $\mu g$ N, respectively.
Figure 3. Rates of autotrophic and heterotrophic nitrification and the respective fraction of these nitrogen transformations emitted as N$_2$O (RN$_2$O$_{a}$ and RN$_2$O$_{h}$, respectively). Note that red diagonal lines on the y-axis denote axis breaks to show changes of RN$_2$O at lower magnitude for RN$_2$O$_{a}$ from the sandy clay loam, and RN$_2$O$_{h}$ for the clay and the loam.
mainly via nitrification mediated pathways (Bollmann and Conrad 1998, Bateman and Baggs 2005). Partitioning of N$_2$O in response to wetting in this study shows however denitrification as the main N$_2$O source even at WFPS $\leq$ 60% in the clay and the loam, and accounting for $>$30% of N$_2$O emissions in sandy CL. Dry periods induce the build-up of N bearing substrates in grassland soils (Harris et al 2021). Microbial activity increases rapidly upon wetting of dry soils (Congreves et al 2018), resulting in increased microbial O$_2$ consumption, which creates favourable conditions for denitrification. This effect is likely to be more pronounced in high organic C pasture soils with high microbial activity (Friedl et al 2020), where the release of solutes from microbial cells (Schimel et al 2007) and the decomposition of the microbial necromass (Kieft et al 1987) supplies low C:N substrate in response to wetting. Source partitioning of N$_2$O demonstrated in the study here integrates the above-mentioned effects of wetting on N$_2$O production, creating conditions conducive for denitrification even at low soil water contents.

The magnitude of N$_2$O$_4$ emissions followed the same pattern as cumulative N$_2$O emissions (figure 1, table 2). In the clay and the sandy CL, N$_2$O$_4$ increased exponentially with WFPS, with peak N$_2$O emissions $>5$ µg N$_2$O-N emitted from the clay. In the loam however, the exponential increase was only observed up to 80% WFPS, with a subsequent decrease in N$_2$O$_4$ emissions. Emissions of N$_2$O are generally expected to decrease when soil moisture reaches saturation, as hypoxic conditions favour the reduction of N$_2$O to N$_2$ (Morley and Baggs 2010). The exponential increase of N$_2$O$_4$ in the clay and sandy CL however denotes residual O$_2$ in the soil matrix and a delay in the development of anaerobiosis in these soils. The exponential increase in N$_2$O emissions in response to wetting and fertilisation may be further caused by high NO$_3^-$ availability, promoting preferential NO$_3^-$ reduction, and thus limiting the reduction of N$_2$O to N$_2$ (Senbayram et al 2019, Friedl et al 2020). The effect of wetting is amplified by the high labile C availability in pasture soils, supplying an energy source to heterotrophic denitrifiers, but also increasing microbial O$_2$ consumption (Azam et al 2002, Meyer et al 2010) and the subsequent formation of anaerobic microsites. The loam had the highest labile C availability across soils, explaining the decrease of N$_2$O$_4$ emissions $>80%$ WFPS as a consequence of increased microbial O$_2$ consumption. Limited O$_2$ availability in the soil matrix induces NO$_3^-$ consumption by DNRA (Friedl et al 2018, Putz et al 2018), and the reduction of N$_2$O to N$_2$, both limiting N$_2$O$_4$ emissions. The effect of labile C availability on denitrification is further reflected in the fraction of denitrification (N$_2$ + N$_2$O) lost as N$_2$O (RN$_2$O$_4$) (figure 2). Across soils, RN$_2$O$_4$ followed a similar pattern decreasing at WFPS levels $\leq$60%, yet the rate of decrease increased with labile C concentration, with lowest values for RN$_2$O$_4$ observed from the loam near saturation. These findings highlight the soil specific response of N$_2$O formation via denitrification in pasture soils and show the combined effect of soil moisture and microbial O$_2$ consumption on N$_2$O$_4$ production.

Co-denitrification was negligible for N$_2$O production in this study. Large N$_2$O fluxes from co-denitrification of $>$80 mg N$_2$O–N m$^{-2}$ d$^{-1}$ have been reported from urine patches (Selbie et al 2015), while NH$_4$NO$_3$ additions comparable to the study here induced only minor N$_2$O fluxes ($\leq$5% of cumulative N$_2$O) (Jansen-Willems et al 2016). The production of N$_2$O via co-denitrification maybe, therefore, closely linked to the chemical and biological reactions triggered by high urine/urea N deposition in pasture soils (Spott et al 2011, Breuillin-Sessoms et al 2017, Clough et al 2020).

4.2. Production of N$_2$O from autotrophic and heterotrophic nitrification in response to wetting

The $^{15}$N$_2$O pool mixing model used in this study attributes N$_2$O without $^{15}$N label to the unlabelled organic N pool, and therefore to heterotrophic nitrification. Other pathways of N$_2$O production using organic N substrates include chemo and co-denitrification (Butterbach-Bahl et al 2013). These pathways form however hybrid N$_2$O by combining labelled NO$_2$ with organic N compounds at natural abundance, differing in their isotopic composition from N$_2$O$_4$ at natural abundance.

The observed heterotrophic nitrification rates are amongst the highest reported from agricultural soils (Chen et al 2015, Zhang et al 2015) and exceed reported rates from grassland soils (Rütting et al 2010, Müller et al 2014, Jansen-Willems et al 2016). Production of N$_2$O$_4$ is thought to occur via the oxidation of reduced N in organic matter to NO$_3^-$ and NO$_2^-$, and the subsequent reduction to N$_2$O (Braker and Conrad 2011). Similar to nitrifier-denitrification, the process links an aerobic metabolism with denitrification (Blagodatsky et al 2006), and is likely more adapted to fluctuating redox conditions in soils triggered by wetting events. This is consistent with the production of N$_2$O$_4$, remaining constant or increasing with increasing WFPS. Peak emissions of N$_2$O$_4$ denote 80% WFPS as optimum across soils, with subsequent emissions decreasing. Production of N$_2$ via heterotrophic nitrification has been suggested in waste-water treatment reactors (Zhao et al 2012), its significance for soil gas exchange remains however unknown. Regardless of its source, N$_2$O is more likely reduced to N$_2$ at high soil water content, due to low O$_2$ availability and prolonged retention of N$_2$O in the soil (Hansen et al 2014), explaining the decrease of N$_2$O$_4$ at soil moisture contents $>$80% WFPS.
The fraction $RN_2O_3$ shows a decoupling between the respective rates of NO$_3^-$ production and magnitude of N$_2$O emitted derived from autotrophic nitrification. Autotrophic nitrification rates were either $b<5\ \mu$g NO$_3^-$/N g$^\text{-1}$ soil or decreased below this threshold when WFPS > 80% (figure 3), denoting the sensitivity of NO$_3^-$ production by autotrophic nitrifiers to increasing anaerobiosis. Emissions of N$_2$O however increased in the clay and the loam, resulting in an exponential response of $RN_2O_3$ to increasing soil moisture. Nitrification as a source of N$_2$O has been traditionally attributed to aerobic conditions in agricultural soils, yet the response to wetting shown here implies increasing N$_2$O production along the ammonia oxidising pathway under O$_2$ limited conditions. The response of $RN_2O_3$ to wetting is consistent with the reported increase of $RN_2O_3$ with decreasing O$_2$ availability (Zhu et al 2013), which has been attributed to nitrifier-denitrification, i.e. the ability of ammonia oxidisers to denitrify (Wragge et al 2001, Prosser et al 2020). The $^{15}$N$_2O$ pool mixing model used in our study links N$_2$O production to the respective N source pools and this representation of N$_2$O production correlates with the one in the HIP model. The N substrate supplying pathway may however differ from the microbial process of N$_2$O production and can respond differently to wetting. Besides autotrophic nitrifiers, heterotrophic denitrifiers are also able to use NO$_3^-$ produced via autotrophic nitrification as a substrate for N$_2$O production (Liu et al 2013), which could also explain continued emissions of N$_2$O at high WFPS. High WFPS and therefore reduced soil gas diffusivity is also likely to have delayed N$_2$O surface emissions, which were produced when residual O$_2$ was still abundant, facilitating autotrophic nitrification at microsites. The contribution of nitrifier-denitrification and denitrification to N$_2$O production has been debated (Bakken and Frostegård 2017) as methodological constraints hinder accurate N$_2$O partitioning (Prosser et al 2020). Analogue to biogeochemical models, the source partitioning model used in the study here summarises these processes under N$_2$O emissions according to the source of the N substrate, and suggests that heterotrophic and/or autotrophic denitrification of NO$_2^-$ derived from nitrification drives the increase of $RN_2O_3$ in response to wetting.

The fraction $RN_2O_h$ did not follow a common pattern across soils, reflecting vastly different N$_2$O and NO$_3^-$ production via heterotrophic nitrification. Production of N$_2$O via heterotrophic nitrification has been shown to increase with decreasing soil pH (Zhang et al 2018b), which is consistent with the observed negative correlation of N$_2$O$_h$ and soil pH in the study presented here. The highest emissions of N$_2$O$_h$ were observed from the sandy CL, together with the lowest rates of heterotrophic nitrification (figure 3), implying different factors driving NO$_3^-$ and related N$_2$O$_h$ production. The sandy CL has the lowest pH across soils and has been characterised as a pasture soil under extensive management (De Rosa et al 2020), receiving less mineral N fertiliser inputs than the clay and the loam. Heterotrophic nitrifiers comprise a large variety of phylogenetically unrelated bacteria and fungi (Braker and Conrad 2011) and fungi have been shown to dominate heterotrophic NO$_3^-$ production under more acidic conditions (Zhu et al 2015). Specific adaptation of the soil microbial community to soil chemical properties and N fertilisation (Zhang et al 2018a) may explain the differences in partitioning of N$_2$O production between heterotrophic and autotrophic nitrification with important implication for practical N$_2$O abatement strategies: Nitrification inhibitors such as 3,4-dimethylpyrazole phosphate (DMPP) inhibit the activity of the ammonia monoxygenase (AMO), facilitating ammonia oxidation to hydroxylamine. Heterotrophic nitrification by fungi is however thought to lack AMO (Wood 1990), suggesting that nitrification inhibitors are ineffective in mitigating N substrate supply for N$_2$O production via this pathway. The contribution of heterotrophic nitrification to N$_2$O production in response to wetting across soils shows that this pathway is not restricted to strong acidic conditions and needs to be considered when managing N substrate availability for N$_2$O formation in intensively managed pasture soils.

Fractions of N$_2$O emitted from autotrophic and heterotrophic nitrification differed in their response to wetting. However, the fraction of overall nitrification emitted as N$_2$O increased exponentially across all soils, with an inflection point between 60 and 80% WFPS. These findings demonstrate the importance of both processes for N substrate availability for N$_2$O production. The increase of $RN_2O_a$ however suggests denitrification of nitrified N as the process of N$_2$O production, driven by the rapid depletion of soil O$_2$. Our findings are consistent with the response of RN$_2O_a$ to decreasing O$_2$ availability (Khalil et al 2004, Zhu et al 2013), and confirm the exponential relationship implemented in models such as NOE (Hénault et al 2005) and DAYCENT (Yang et al 2017). The use of a constant for $RN_2O_h$ independent of soil WFPS is however likely to underestimate N$_2$O derived from nitrification when simulating large rainfall events.

4.3. The HIP

The response of N$_2$O production demonstrates an exponential increase of N$_2$O emissions with the amount of wetting, dominated by denitrification across pasture soils. The immediate reduction of N$_2$O to N$_2$ even at low soil water content denotes increased microbial activity and therefore O$_2$ consumption following the wetting of dry pasture soils, driving the N$_2$O:N$_2$ ratio towards N$_2$. The inclusion of the organic N pool into the $^{15}$N$_2O$ mixing model revealed a significant contribution of heterotrophic nitrification to N$_2$O production, which appears to
be less sensitive to increasing amounts of wetting than autotrophic nitrification. Losses of $\text{N}_2\text{O}_3$ and $\text{N}_2\text{O}_5$ suggest that build up and sudden release of C and N substrate upon wetting stimulates $\text{N}_2\text{O}$ production via denitrification and via the oxidation of organic N. Continuous $\text{N}_2\text{O}$ emissions derived from autotrophic nitrification even at high WFPS highlight the decoupling between the pipe and the hole, i.e. between the rate of the N transformation and the respective fraction lost as $\text{N}_2\text{O}$. This is shown by the exponential increase of $\text{RN}_2\text{O}_3$ with increasing soil water content, suggesting denitrification of nitrified $\text{NO}_3^-$ causing $\text{N}_2\text{O}$ losses from nitrification mediated pathways at high soil water content. The consideration of the $\text{NO}_3^-$ pool as a central N pool in models such as in LDNDC may therefore help to accurately simulate $\text{N}_2\text{O}$ production via nitrification mediated pathways, and further research tracing $^{15}\text{N}$ in the $\text{NO}_3^-$ pool is needed to deliver experimental evidence and validation data for this approach. Our findings demonstrate that the proportion of nitrified N lost as $\text{N}_2\text{O}$ ($\text{R}_{\text{N}_2\text{O}_3}$) is not constant as assumed in some models but increases exponentially with the degree of wetting. These results provide important experimental evidence for the relationship of soil water with $\text{N}_2\text{O}$ production and corroborate the exponential response of $\text{R}_{\text{N}_2\text{O}_3}$ in models such as SWAT and NOE. Importantly, the $\text{R}_{\text{N}_2\text{O}_3}$ response curves established in our study combine physical, chemical, and biological effects of wetting on $\text{N}_2\text{O}$ production pathways from pasture soils, and their implementation in modelling approaches may help to increase model performance when simulating drying and wetting cycles. The large contribution of heterotrophic nitrification to $\text{N}_2\text{O}$ production suggests an opportunity to improve models by adding further pathways of $\text{N}_2\text{O}$ production. The resulting complexity however needs to be weighed against potential benefits, ensuring accurate $\text{N}_2\text{O}$ forecasting for intensively managed pasture systems.

**Data availability statement**

The data that support the findings of this study are available upon reasonable request from the authors.

**Acknowledgments**

This study was supported by funding from the Australian Government Department of Agriculture and Water Resources as part of its Rural R&D for Profit programme, and Dairy Australia. Clemens Scheer acknowledges funding from the German Federal Ministry of Education and Research (BMBF) under the ‘Make our Planet Great Again—German Research Initiative’, Grant No. 306060, implemented by the German Academic Exchange Service (DAAD). This study was carried out in collaboration with the German Science Foundation (DFG) research unit DASIM (FOR 2337) ‘Denitrification in Agricultural Soils: Integrated control and Modelling at various scales’. The data reported in this paper were obtained at the Central Analytical Research Facility (CARF) operated by the Institute of Future Environments (QUT). Access to CARF is supported by generous funding from the Science and Engineering Faculty (QUT).

**ORCID iDs**

Johannes Friedl  [https://orcid.org/0000-0003-0468-916X](https://orcid.org/0000-0003-0468-916X)

Clemens Scheer  [https://orcid.org/0000-0001-5396-2076](https://orcid.org/0000-0001-5396-2076)

Daniele De Rosa  [https://orcid.org/0000-0002-0441-7722](https://orcid.org/0000-0002-0441-7722)

Peter R Grace  [https://orcid.org/0000-0003-4136-4129](https://orcid.org/0000-0003-4136-4129)

David W Rowlings  [https://orcid.org/0000-0002-1618-9309](https://orcid.org/0000-0002-1618-9309)

**References**


Bakken L R and Frostegård Å 2017 Sources and sinks for N2O, can microbiologist help to mitigate N2O emissions? Environ Micribiol 19 4801–3


Bogataiky S A, Kesik M, Papen H and Butterbach-Bahl K 2006 Production of NO and $\text{N}_2\text{O}$ by the heterotrophic nitrifier alcaligenes faecalis parafaecalis under varying conditions of oxygen saturation Geomicrobiol. J. 23 165–76


Borken W and Matzner E 2009 Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils Glob. Change Biol. 15 808–24


Butterbach-Bahl K, Baggs E M, Dannenmann M, Kiese R and Zechmeister-Boltenstern S 2013 Nitrous oxide emissions...
from soils: how well do we understand the processes and their control? Phil. Trans. R. Soc. B 368 1621, p201201222
Clough T J et al 2017 Influence of soil moisture on codenitrification fluxes from a urea-affected pasture soil Plant Rep. 7 2185
Decock C and Six J 2013 How reliable is the intramolecular distribution of 15N in N₂O to source partition N₂O emitted from soil? Soil Biol. Biochem. 65 114–27
Fuchs K et al 2020 Multimodel evaluation of nitrous oxide emissions from an intensively managed grassland J. Geophys. Res.-Biogeo. 125 e2019JG005261
Harris E et al 2021 Denitrifying pathways dominate nitrous oxide emissions from managed grassland during drought and rewetting Sci. Adv. 7 eab67118
IUSS W G 2015 World reference base for soil resources 2014 (update 2015). International soil classification system for naming soils and creating legends for soil maps World Soil Resources Reports 106
Khalil K, Mary B and Renault P 2004 Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O2 concentration Soil Biol. Biochem. 36 687–99
Kieft T L, Soroker E and Firestone M K 1987 Microbial biomass response to a rapid increase in water potential when dry soil is wetted Soil Biol. Biochem. 19 119–26
Li C, Frolking S and Butterbach-Bahl K 2005 Carbon sequestration in arable soils is likely to increase nitrous oxide emissions, offsetting reductions in climate radiative forcing Clim. Change 72 321–38
Estimation of parameters in complex 15N tracing models by
Monte Carlo sampling Soil Biol. Biochem. 39 715–26
Müller C, Stevens R and Laughlin R 2004 A 15 N tracing model to
analyse N transformations in old grassland soil Soil Biol.
Biochem. 36 619–32
Mumford M T, Rowlings D W, Scheer C, De Rosa D and
Grace P R 2019 Effect of irrigation scheduling on nitrous
oxide emissions in intensively managed pastures Agric.
Ecosyst. Environ. 272 126–34
Murphy B F and Ribbe J 2004 Variability of southeastern
Queensland rainfall and climate indices Int. J. Climatol. 24
703–21
Nepciçova M, Anex R P, Fienen M N, Del Grosso S J,
Castellano M J, Sawyer J E, Igelb J, Pantoja J L and
Barker D W 2015 Understanding the DayCent model:
calibration, sensitivity, and identifiability through inverse
modeling Model. Softw. 66 110–30
Prosser J J, Hink L, Gubry-Rangin C and Nicol G W 2020 Nitrous
oxide production by ammonia oxidizers: physiological
diversity, niche differentiation and potential mitigation
strategies Glob. Change Biol 26 103–18
Putz M, Schleusner P, Rütting T and Hallin S 2018 Relative
absence of denitrifying and DNRA bacteria and their
activity determine nitrogen retention or loss in agricultural
soil Soil Biol. Biochem. 123 97–104
oxide (N₂O): the dominant ozone-depleting substance
emitted in the 21st century Science 326 123–5
Rex D, Clough T J, Richards K G, Condron L M, De Klein C A M,
Morales S E and Lanigan G J 2019 Impact of nitrogen
compounds on fungal and bacterial contributions to
codenitrification in a pasture soil Sci. Rep. 9 13371
Rowlings D W, Grace P R, Scheer C and Liu S 2015 Rainfall
variability drives interannual variation in N₂O emissions
from a humid, subtropical pasture Sci. Total Environ. 512–513 8–18
Rütting T, Clough T J, Müller C, Lieffering M and Newton P C.
2010 Ten years of elevated atmospheric carbon dioxide alters
soil nitrogen transformations in a sheep-grazed pasture
Glob. Change Biol. 16 2530–42
Scheer C, Rowlings D W, Firrell M, Deuter P, Morris S and
Grace P R 2014 Impact of nitrification inhibitor (DMPP) on
soil nitrous oxide emissions from an intensive broccoli
production system in sub-tropical Australia Soil Biol.
Biochem. 70 233–51
Schimel J, Baltzer T C and Wallenstein M 2007 Microbial
stress-response physiology and its implications for
ecosystem function Ecology 88 1386–94
Selbie D R et al 2015 Confirmation of co-denitrification in grazed
grassland Sci. Rep. 5 17361
Senbayram M, Budai A, Bol R, Chadwick D, Marton L,
Gündogán R and Wu D 2019 Soil NO3− level and O2
availability are key factors in controlling N₂O reduction to
N2 following long-term liming of an acidic sandy soil Soil
Biol. Biochem. 132 165–73
Spott O, Russow R and Stange C F 2011 Formation of hybrid N₂O
and hybrid N₂ due to codenitrification: first review of a
barely considered process of microbially mediated
Stark J M and Hart S C 1996 Diffusion technique for preparing
salt solutions, Kjeldahl digests, and persulfate digests for
Stein L Y 2011 Heterotrophic nitrification and nitrifier
95–114
Stevens R J, Laughlin R J, Burns L C, Arah J R M and Hood R C
1997 Measuring the contributions of nitrification and
denitrification to the flux of nitrous oxide from soil Soil Biol.
Biochem. 29 139–51
Stott K J and Gourley C J P 2016 Intensification, nitrogen use and
recovery in grazing-based dairy systems Agric. Syst.
144 101–12
Van Lent J, Hergoualc’h K and Verchot L 2015 Reviews and
syntheses: soil N₂O and NO emissions from land use and
land use change in the tropics and sub-tropics: a
meta-analysis Biogeosciences 12 7299–313
2003 Estimating active carbon for soil quality assessment: a
simplified method for laboratory and field use Am. J. Altern.
Agric. 18 3–17
Wood P 1990 Autotrophic and heterotrophic mechanisms for
ammonia oxidation Soil Use Manage. 6 78–79
Wood S 2015 Package ‘mgcv’ R package version pp 1–7
Wrange N, Veldho G L, Van Beusichem M L and Oenema O 2001
Role of nitrifier denitrification in the production of nitrous
oxide Soil Biol. Biochem. 33 1723–32
Wragé-Mönnig N, Horn M A, Well R, Müller C, Veldho G and
Oenema O 2018 The role of nitrifier denitrification in the
production of nitrous oxide revisited Soil Biol. Biochem. 123
A3–A16
Yang Q, Zhang X, Abhra M, Del Grosso S, Robertson G P and
Chen J 2017 Enhancing the soil and water assessment tool
model for simulating N₂O emissions of three agricultural
systems Ecosyst. Health Sustain. 3 e01259
Yoon S, Song B, Phillips R L, Chang J and Song M J 2019
Ecological and physiological implications of nitrogen oxide
reduction pathways on greenhouse gas emissions in
agroecosystems FEMS Microb. Ecol. 95 e0666
Yu L et al 2020 What can we learn from N₂O isotope
data?—Analytics, processes and modelling Rapid Commun.
Mass Spectrom. 34 e6858
Zaman M et al 2021 Isotopic techniques to measure N₂O, N₂ and
their sources Measuring Emission of Agricultural Greenhouse
Gases and Developing Mitigation Options Using Nuclear
and Related Techniques: Applications of Nuclear Techniques for
GHGs ed M Zaman, L Heng and C Müller (Cham: Springer
International Publishing) pp 213–301
Zhang J, Müller C and Cai Z 2015 Heterotrophic nitrification of
organic N and its contribution to nitrous oxide emissions in
soils Soil Biol. Biochem. 84 199–209
Zhang Y, Ding H, Zheng X, Ren X, Cardenas L, Carswell A and
Miselisbrot T 2018a Land-use type affects N₂O production
pathways in subtropical acidic soils Environ. Pollut. 237
237–43
Zhang Y, Zhao W, Cai Z, Müller C and Zhang J 2018b
Heterotrophic nitrification is responsible for large rates of
N₂O emission from subtropical acid forest soil in China Eur.
J. Soil Sci 69 646–54
Zhao B, An Q, He Y L and Guo J S 2012 N₂O and N₂ production
during heterotrophic nitrification by Alcaligenes faecalis
strain NR Bioreour. Technol. 116 379–85
Fungi-dominated heterotrophic nitrification in a subtropical
forest soil of China J. Soils Sediments 15 705–9
Zhu X, Burger M, Doane T A and Horwath W R 2013 Ammonia
oxidation pathways and nitrifier denitrification are
significant sources of N₂O and NO under low
oxygen availability Proc. Natl Acad. Sci. USA 110 6328–33