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

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Abstract

Rainfall and irrigation trigger large pulses of the powerful greenhouse gas N_2O from intensively managed pastures, produced via multiple, simultaneously occurring pathways. These N_2O pulses can account for a large fraction of total N_2O losses, demonstrating the importance to determine magnitude and source partitioning of N_2O under these conditions. This study investigated the response of different pathways of N_2O production to wetting across three different textured pasture soils. Soil microcosms were fertilised with an ammonium nitrate (NH_4NO_3) solution which was either single or double ^{15}N labelled, wetted to four different water-filled pore space (WFPS) levels, and incubated over two days. The use of a ^{15}N pool mixing model together with soil N gross transformations enabled the attribution of N_2O to specific pathways, and to express N_2O emissions as a fraction of the underlying N transformation. Denitrification and nitrification mediated pathways contributed to the production of N_2O in all soils, regardless of WFPS. Denitrification was the main pathway of N_2O production accounting for >50% of cumulative N_2O emissions even at low WFPS. The contribution of autotrophic nitrification to N_2O 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_2O, increasing exponentially with WFPS, while the percentage of denitrified N emitted as N_2O decreased, providing critical information for the representation of N_2O/WFPS relationships in simulation models. Our findings demonstrate that the wetting of pasture soils promotes N_2O 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_2O emissions should be considered when developing N_2O abatement strategies, seeking to reduce N_2O emissions in response to rainfall and irrigation from intensively managed pastures.

1. Introduction

Pasture soils are a major source of nitrous oxide (N_2O), a powerful greenhouse gas with a global warming potential 298 times higher than that of carbon dioxide (CO_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_2O from pasture soils. In intensively managed pasture systems such as dairy pastures, N substrate availability for N_2O 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_2O 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_2O 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_2O productions under these conditions are highly uncertain despite an increasingly well-defined mechanistic understanding of N_2O production (Bakken and Frostegård 2017, Yoon *et al* 2019), and the representation of N_2O 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_2O production pathways, the reduction of N_2O 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_2O emissions are depicted as the fraction RN_2O of the underlying N transformation. This conceptual framework is widely used in simulation models such as DAYCENT (Necpálová *et al* 2015), DNDC (Li *et al* 2000), LDNDC (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_2O, 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_2O formation to these pools, and the respective N transformations, i.e. nitrification and denitrification. Following the HIP model, N_2O 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, (Wrage-Mönnig *et al* 2018), while NO_3^- is regarded as the sole source pool of N_2O 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_2O 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_2O 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_2O production in biogeochemical models, recommending its use to establish the contribution of specific N transformation to N_2O production.

Two pathways of N_2O production linked to organic N pool have recently gained more attention due to the inclusion of the organic N pool in $^{15}N_2O$ 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_2O via heterotrophic nitrification is thought to occur via the oxidation of organic N to NO_2^- and its subsequent reduction to N_2O (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_2O production from organic N compounds only. Besides classic denitrification, co-denitrification can also contribute to N_2O production, forming hybrid N_2O 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_2O from temperate pastures has demonstrated the significance of both heterotrophic nitrification (Müller *et al* 2014, Jansen-Willems *et al* 2016, Moser *et al* 2018) and co-denitrification (Selbie *et al* 2015, Rex *et al* 2019) for N_2O production, yet their significance in response to different degrees of wetting remains largely unknown. More importantly, assessments of N_2O production pathways in response to soil water content (Bateman and Baggs 2005, Mathieu *et al* 2006, Loick *et al* 2021) mostly lack data on N_2O reduction to N_2. This hinders the quantification of overall denitrification, and thus the assessment of denitrification as source and sink of N_2O upon wetting.

The aim of this study was therefore to establish the response and significance of nitrification and denitrification mediated pathways of N_2O 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_2O. We combined $^{15}N_2O$ analysis and a $^{15}N_2O$ pool mixing model with soil N transformation and N_2 data presented in Friedl *et al* (2018), allowing the calculation of the contribution of nitrification and denitrification ($N_2 + N_2O$) emitted as N_2O 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_2O emitted from denitrification (RN_2O_d), the magnitude of overall denitrification (Del Grosso *et al* 2020), and the fraction of N_2O

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

Soil property	Clay	Loam	Sandy clay loam
Site	Casino	Gympie	Kerry
Latitude	−28.865	−26.19	−28.109
Longitude	152.874	152.74	153.031
Mean annual rainfall	1107 mm	1127 mm	906.7 mm
Soil type (ASC)	Vertosol	Dermosol	Tenosol
Soil type (FAO)	Pellic Vertisol	Ferric Acrisol	Mollic Fluvisol
Texture (USDA)	Clay	Loam	Sandy clay loam
pH (water, 1:5)	6.3	6.1	5.9
Organic Carbon (%)	4.2	4.9	4.1
Total Nitrogen (%)	0.36	0.5	0.4
C:N ratio	11.4	9.8	10.4

emitted via nitrification mediated pathways (RN_2O_n) (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 N_2O 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.

2.1. ^{15}N 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 ^{15}N labelling approach combined with a ^{15}N tracing model (Friedl *et al* 2018). In the study presented here, we used additional $^{15}N_2O$ gas analysis together with gross N transformation data presented in Friedl *et al* (2018) to attribute N_2O losses to specific N_2O production pathways.

The experimental setup is described in Friedl *et al* (2018). Briefly, soil collected from the three pasture sites was partially airdried (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 NH_4NO_3 solution containing the equivalent of 35 μg N g^{-1} soil was applied to each microcosm, either single ($NH_4{}^{15}NO_3$) labelled (a) or double ($^{15}NH_4{}^{15}NO_3$) 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 N_2O and CO_2 analysis by gas chromatography (Shimadzu GC-2014). Gas samples were analysed for isotopologues of N_2O ($^{14}N^{14}N$, $^{14}N^{15}N$ and $^{15}N^{15}N$, and $^{14}N^{14}N^{16}O$) 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 $^{15}N_2O$.

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 ^{15}N treatment were used for $t = 0$ extractions, and all soil microcosms (treatment a and b) were extracted at $t = 2$ d. The ^{15}N enrichment of the soil mineral N pool was determined by the diffusion method (Stark and Hart 1996). Gross N transformations were quantified using the ^{15}N 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_2O and CO_2, and N_2O source partitioning

Fluxes of N_2O 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_2O was then used to attribute N_2O 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_2O 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 ($^{45}I/^{44}I$) and ^{46}R ($^{46}I/^{44}I$) to be calculated. Possible N_2O species include also ^{47}R and ^{48}R and were calculated as

$$^{47}R = \left(^{15}R\right)^2 * {}^{17}R + 2 * {}^{15}R * {}^{18}R \quad (1)$$

$$^{48}R = {}^{18}R * \left(^{15}R\right)^2 \quad (2)$$

with ^{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_2O is then calculated as

$$a_{N_2O} = \left(^{45}R + 2 * {}^{46}R - {}^{17}R - 2 * {}^{18}R\right) / \left(2 + 2 * {}^{45}R + 2 * {}^{46}R\right). \quad (3)$$

Emissions of N_2O 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_n, heterotrophic nitrification (O_{Nrec}), defined as the oxidation of organic N at natural abundance a_o, and hybrid formation of N_2O attributed to co-denitrification. Hybrid N_2O is formed by one N atom from the NO_3^- pool and one from the organic N pool. The ^{15}N fraction of N_2O is given by equation (4) as

$$a_{N_2O} = d * a_d + n * a_n + h * a_o + \mathrm{cd} * (0.5 * (a_d + a_o)) \quad (4)$$

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_2O emitted via denitrification, autotrophic nitrification, heterotrophic nitrification and co-denitrification, respectively. Daily values for a_d and a_n 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_2O, using all possible combinations between the different replicates of treatment (a) and (b) ($n = 16$). The fraction h was calculated as

$$h = 1 - d - n - \mathrm{cd} \quad (5)$$

with all the N_2O assumed to come from one of the four N_2O 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 all the combinations. Multiplying the N_2O flux with d, n, h, and cd gave the amount of N_2O emitted via denitrification (N_2O_d), autotrophic nitrification (N_2O_a), heterotrophic nitrification (N_2O_h) and co-denitrification (N_2O_{cd}), respectively. Emissions of N_2O produced by all nitrification mediated pathways (N_2O_n) were calculated as the sum of N_2O_a and N_2O_h. Following the hole in the pipe model, the fraction of N_2O lost via denitrification was expressed as

$$RN_2O_d = \frac{N_2O_d}{\mathrm{Den}} \quad (6)$$

where Den was overall denitrification, calculated using the product ratio of denitrification $N_2O/(N_2 + N_2O)$ obtained in Friedl *et al* (2018) (treatment c) and the N_2O_d from treatment (a) and (b). The RN_2O factors for autotrophic nitrification (O_{NH4}) and heterotrophic nitrification (O_{Nrec}) were calculated accordingly using equations (7) and (8):

$$RN_2O_a = \frac{N_2O_n}{O_{NH4}} \quad (7)$$

$$RN_2O_h = \frac{N_2O_h}{O_{Nrec}}. \quad (8)$$

The factor RN_2O_n for total nitrification was calculated as

$$RN_2O_n = \frac{N_2O_n}{O_{Nrec} + O_{NH4}}. \quad (9)$$

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_2O, N_2O_d, N_2O_{cd}, N_2O_a, N_2O_h, N_2O_n 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_n, 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 μg 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_{cd} 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_h emissions increased with increasing WFPS reaching a plateau at WFPS ⩾ 80%. Heterotrophic nitrification exceeded autotrophic nitrification as an N_2O production pathway in the loam 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_n 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_d) 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 μg NO_3^-N g^{-1} d^{-1} soil and decreased thereafter. Across all soils, RN_2O_n 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 μg NO_3^-N g^{-1} soil day^{-1}, and decreased to less than 2 μg NO_3^-N g^{-1} d^{-1} at 95% WFPS. In the loam, autotrophic nitrification remained <1.5 μg NO_3^-N g^{-1} d^{-1} across all WFPS levels. The fraction RN_2O_a 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_a 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 μg NO_3^-N g^{-1} soil day^{-1} at 95% WFPS, respectively. Heterotrophic nitrification peaked between 60 and 80% WFPS in

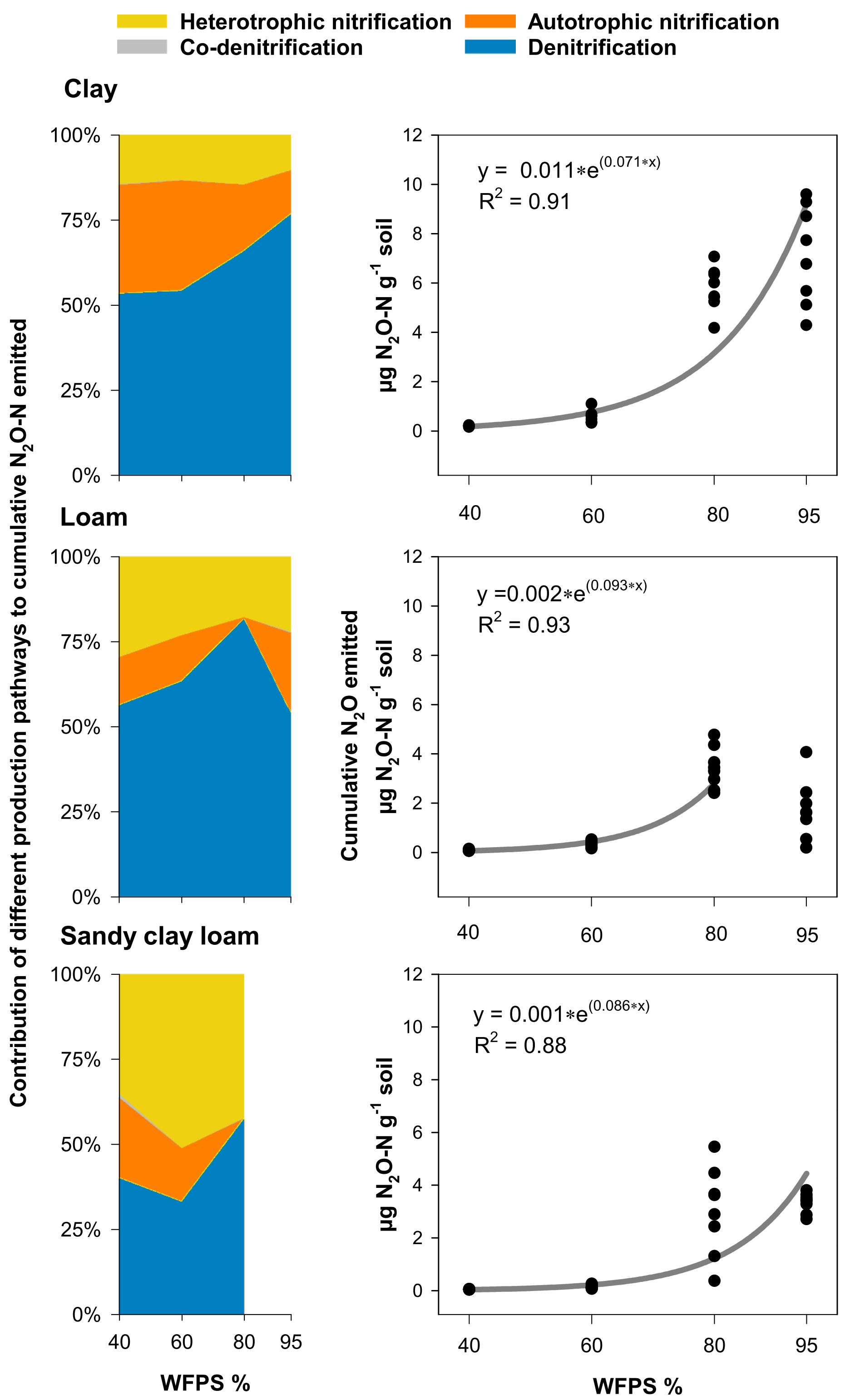

Figure 1. Cumulative N_2O emissions and the contribution of heterotrophic nitrification, autotrophic nitrification, co-denitrification and denitrification to N_2O emissions in response to increasing soil water filled pore space across three different textured pasture soils.

the loam at >than 40 μg NO_3^--N g^{-1} soil day^{-1} and decreased thereafter. Highest values for RN_2O_h 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, RN_2O_h remained below 0.04, peaking at 80 and 95% WFPS, respectively.

3.4. Labile C availability and cumulative CO_2 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),

Table 2. Cumulative N_2O emissions from denitrification (N_2O_d), autotrophic nitrification N_2O_a, heterotrophic nitrification (N_2O_h), total nitrification (N_2O_n) and total N_2O from three different textured pasture soils at four different soil moisture levels and their relative contribution in % to overall N_2O emissions.

	Clay				Loam				Sandy clay loam			
WFPS		↓	↔	%		↓	↔	%		↓	↔	%
N_2O_d N μg N g^{-1}soil												
40%	0.108 ± 0.007	c	A	53	0.048 ± 0.008	c	B	57	0.019 ± 0.002	b	C	40
60%	0.307 ± 0.08	c	A	55	0.207 ± 0.040	c	A	63	0.047 ± 0.011	b	B	33
80%	3.798 ± 0.288	b	A	66	2.795 ± 0.341	b	B	82	1.732 ± 0.47	a	C	57
95%	5.481 ± 0.767	a	A	77	0.927 ± 0.321	a	B	54				
N_2O_a N μg N g^{-1}soil												
40%	0.064 ± 0.004	b	A	32	0.012 ± 0.002	b	B	14	0.011 ± 0.001	b	B	23
60%	0.178 ± 0.034	b	A	32	0.044 ± 0.009	b	B	13	0.022 ± 0.005	a	B	16
80%	1.13 ± 0.104	a	A	20	0.02 ± 0.004	b	B	1	0.003 ± 0.001	c	B	0
95%	0.922 ± 0.129	a	A	13	0.41 ± 0.142	a	B	24				
N_2O_h N μg N g^{-1}soil												
40%	0.029 ± 0.002	b	A	14	0.025 ± 0.004	c	A	30	0.017 ± 0.002	b	B	36
60%	0.073 ± 0.015	b	A	13	0.076 ± 0.015	c	A	23	0.073 ± 0.018	b	A	52
80%	0.838 ± 0.058	a	AB	15	0.612 ± 0.083	a	B	18	1.288 ± 0.354	a	A	43
95%	0.739 ± 0.103	a	A	10	0.381 ± 0.132	b	B	22				
$N_2O_n = N_2O_h + N_2O_a$ N μg N g^{-1}soil												
40%	0.094 ± 0.006	a	A	47	0.037 ± 0.006	b	B	44	0.028 ± 0.003	b	B	60
60%	0.251 ± 0.049	a	A	45	0.12 ± 0.023	b	B	37	0.095 ± 0.022	b	B	67
80%	1.968 ± 0.156	b	A	34	0.632 ± 0.087	a	C	18	1.291 ± 0.355	a	B	43
95%	1.662 ± 0.232	b	A	23	0.791 ± 0.273	a	B	46				
Total N_2O-N μg N g^{-1}soil												
40%	0.202 ± 0.013	c	A	100	0.084 ± 0.014	c	B	100	0.047 ± 0.004	b	C	100
60%	0.559 ± 0.129	c	A	100	0.327 ± 0.063	c	B	100	0.141 ± 0.033	b	B	100
80%	5.769 ± 0.444	b	A	100	3.427 ± 0.414	a	B	100	3.026 ± 0.825	a	B	100
95%	7.148 ± 0.999	a	A	100	1.724 ± 0.596	b	B	100	3.151 ± 0.186	a	B	100

Letters denote homogenous groups (Tuckey HSD ($p < 0.05$)) for cumulative N_2O emissions (μg N g^{-1}soil).
↓ Within a soil type across different WFPS (small letters) for cumulative N_2O emissions (μg N g^{-1}soil).
↔ Across soil types, within a WFPS treatment (capital letters) for cumulative N_2O emissions (μg N g^{-1}soil).

exceeding values for the clay (856.0 ± 39.4 μgC g^{-1} soil) and the sandy CL (701.4 ± 11.7 μgC g^{-1} soil). Cumulative CO_2 emissions (table S2) as a relative measure of heterotrophic soil respiration were highest in the loam, followed by the clay and the sandy CL, regardless of WFPS and were positively correlated ($P < 0.05$) to labile C availability.

4. Discussion

Wetting events trigger pulses of N_2O emissions from soils, accounting for a large proportion of overall N_2O 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 N_2O pulses (Bergstermann *et al* 2011, Harris *et al* 2021). The short-term response of different N_2O production pathways to wetting of pasture soils provides therefore critical information to constrain magnitude and source partitioning of N_2O pulses. This study demonstrates the simultaneous occurrence of N_2O emissions via denitrification and nitrification mediated pathways across three different pasture soils regardless of the amount of wetting. Partitioning of N_2O emission shows the large contribution of heterotrophic nitrification to N_2O production, highlighting the oxidation of organic N as a major source of N_2O from pasture soils. Following the HIP model, the fraction of total nitrification lost as N_2O (RN_2O_n) ranged from 0.001 to 0.04 and increased exponentially with soil WFPS, while the respective fraction of denitrification (RN_2O_d) 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 N_2O 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 N_2O lost from nitrification mediated pathways is driven by the denitrification of nitrified N.

4.1. Production of N_2O from denitrification and co-denitrification in response to wetting

The use of the ^{15}N pool mixing model showed that denitrification dominated N_2O production in all soils (figure 1), accounting for 40%–80% of cumulative N_2O losses. Under oxic conditions (⩽60% WFPS), production of N_2O is assumed to occur

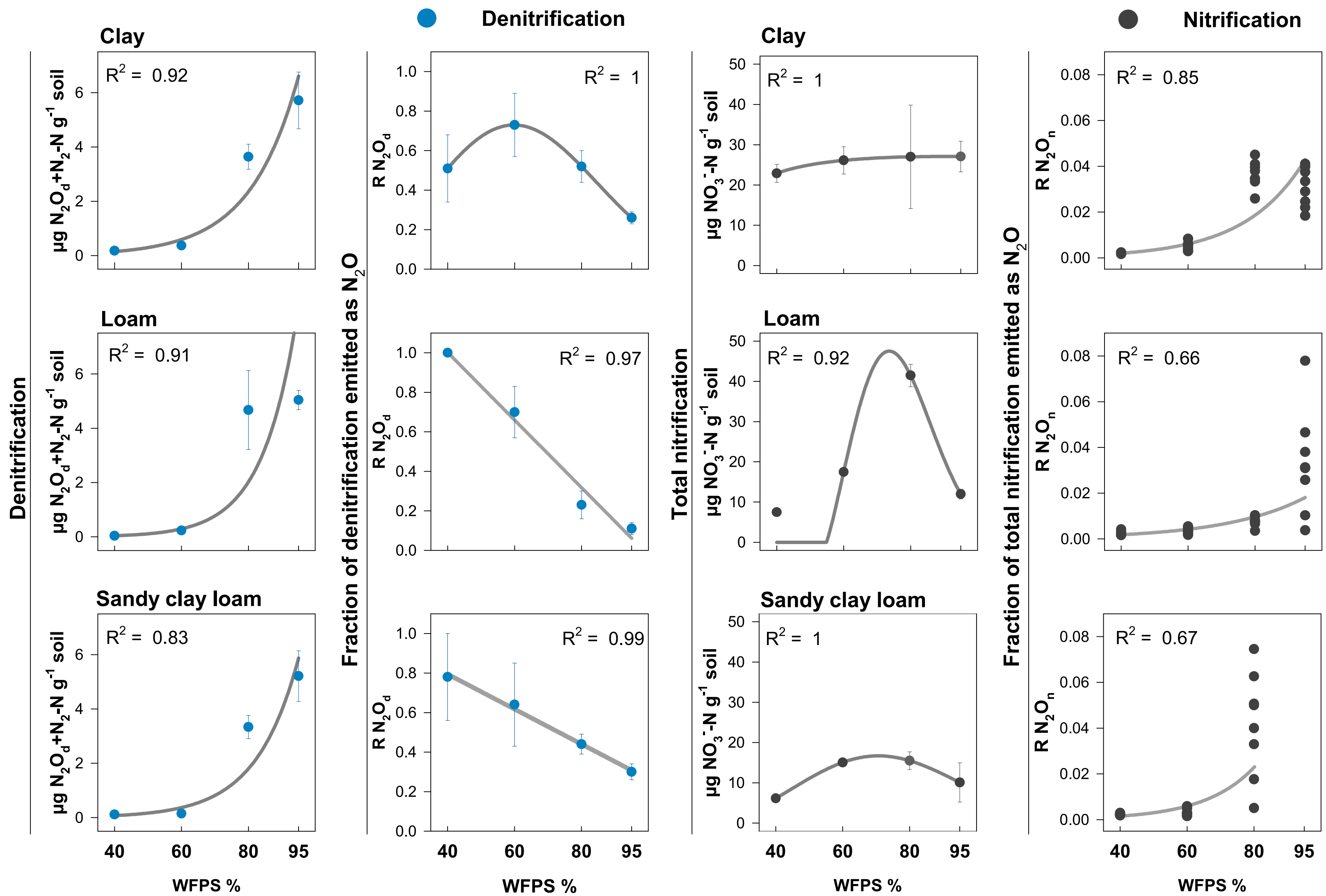

Figure 2. Rates of denitrification ($N_2 + N_2O_d$) and total nitrification and the respective fraction of these nitrogen transformations emitted as N_2O (RN_2O_d and RN_2O_n, respectively). Note the difference in scale for denitrification and nitrification, with maximum values of 7 and 52 μg N, respectively.

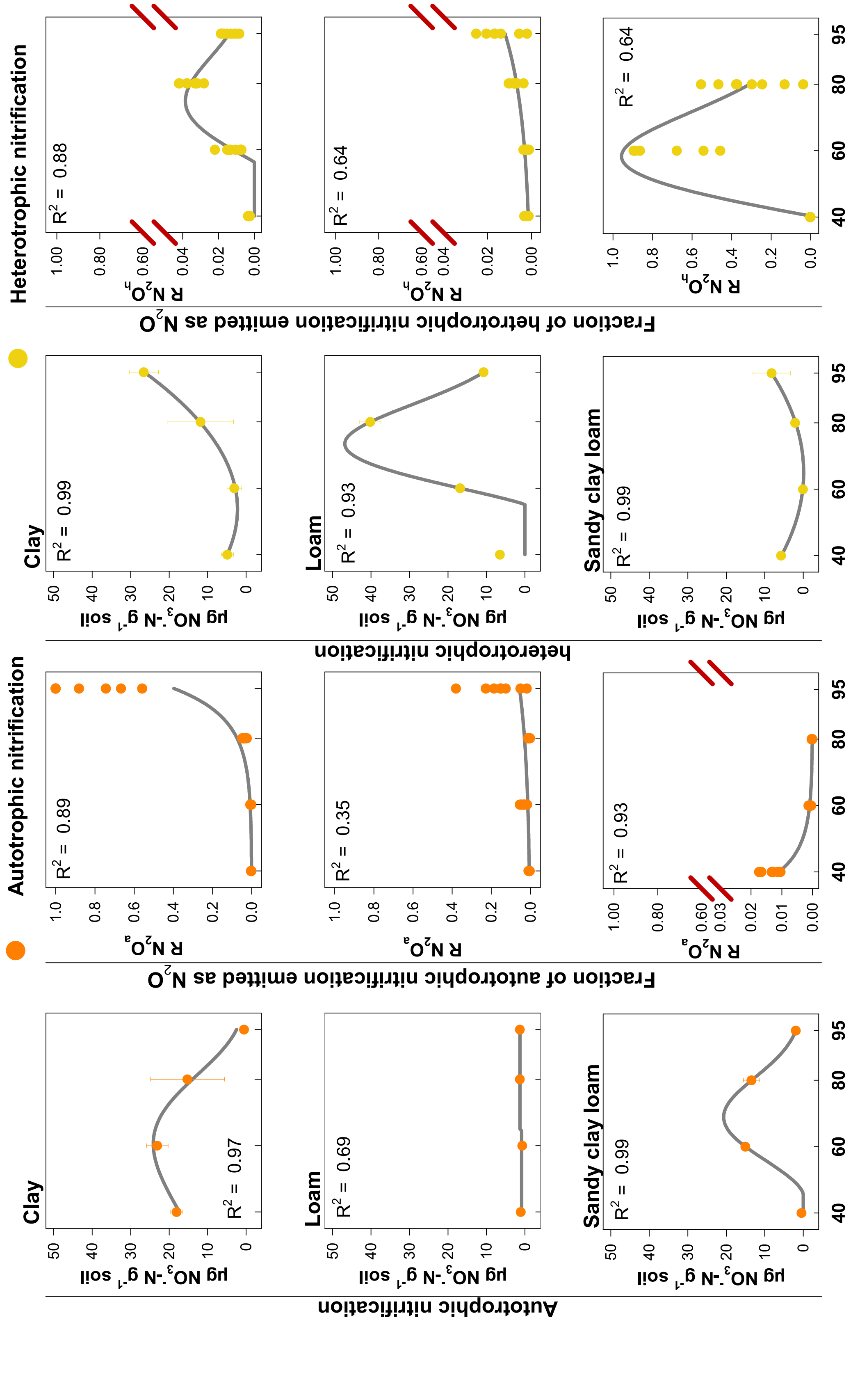

Figure 3. Rates of autotrophic and heterotrophic nitrification and the respective fraction of these nitrogen transformations emitted as N_2O (RN_2O_a and RN_2O_h, respectively). Note that red diagonal lines on the *y* axis denote axis breaks to show changes of R N_2O at lower magnitude for R N_2O_a from the sandy clay loam, and R N_2O_h for the clay and the loam.

mainly via nitrification mediated pathways (Bollmann and Conrad 1998, Bateman and Baggs 2005). Partitioning of N_2O in response to wetting in this study shows however denitrification as the main N_2O source even at WFPS $\leqslant$ 60% in the clay and the loam, and accounting for >30% of N_2O 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_2O demonstrated in the study here integrates the above-mentioned effects of wetting on N_2O production, creating conditions conducive for denitrification even at low soil water contents.

The magnitude of N_2O_d emissions followed the same pattern as cumulative N_2O emissions (figure 1, table 2). In the clay and the sandy CL, N_2O_d increased exponentially with WFPS, with peak N_2O emissions >5 μg N_2O-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_2O_d emissions. Emissions of N_2O are generally expected to decrease when soil moisture reaches saturation, as hypoxic conditions favour the reduction of N_2O to N_2 (Morley and Baggs 2010). The exponential increase of N_2O_d 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_2O 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_2O 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_2O_d 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_2O to N_2, both limiting N_2O_d emissions. The effect of labile C availability on denitrification is further reflected in the fraction of denitrification ($N_2 + N_2O$) lost as N_2O (RN_2O_d) (figure 2). Across soils, RN_2O_d followed a similar pattern decreasing at WFPS levels $\leqslant$60%, yet the rate of decrease increased with labile C concentration, with lowest values for RN_2O_d observed from the loam near saturation. These findings highlight the soil specific response of N_2O formation via denitrification in pasture soils and show the combined effect of soil moisture and microbial O_2 consumption on N_2O_d production.

Co-denitrification was negligible for N_2O production in this study. Large N_2O fluxes from co-denitrification of >80 mg N_2O–N m^{-2} d^{-1} have been reported from urine patches (Selbie *et al* 2015), while NH_4NO_3 additions comparable to the study here induced only minor N_2O_{cd} fluxes ($\leqslant$5% of cumulative N_2O) (Jansen-Willems *et al* 2016). The production of N_2O 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_2O from autotrophic and heterotrophic nitrification in response to wetting

The $^{15}N_2O$ pool mixing model used in this study attributes N_2O without ^{15}N label to the unlabelled organic N pool, and therefore to heterotrophic nitrification. Other pathways of N_2O production using organic N substrates include chemo and co-denitrification (Butterbach-Bahl *et al* 2013). These pathways form however hybrid N_2O by combining labelled NO_2 with organic N compounds at natural abundance, differing in their isotopic composition from N_2O_h 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_2O_h is thought to occur via the oxidation of reduced N in organic matter to NO_2^- and NO_3-, and the subsequent reduction to N_2O (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_2O_h, remaining constant or increasing with increasing WFPS. Peak emissions of N_2O_h 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_2O is more likely reduced to N_2 at high soil water content, due to low O_2 availability and prolonged retention of N_2O in the soil (Hansen *et al* 2014), explaining the decrease of N_2O_h at soil moisture contents >80% WFPS.

The fraction RN_2O_a shows a decoupling between the respective rates of NO_3^- production and magnitude of N_2O emitted derived from autotrophic nitrification. Autotrophic nitrification rates were either b <5 μg NO_3^-N g^{-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_2O_a however increased in the clay and the loam, resulting in an exponential response of RN_2O_a to increasing soil moisture. Nitrification as a source of N_2O has been traditionally attributed to aerobic conditions in agricultural soils, yet the response to wetting shown here implies increasing N_2O production along the ammonia oxidising pathway under O_2 limited conditions. The response of RN_2O_a to wetting is consistent with the reported increase of RN_2O_a 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 (Wrage *et al* 2001, Prosser *et al* 2020). The $^{15}N_2O$ pool mixing model used in our study links N_2O production to the respective N source pools and this representation of N_2O production corresponds with the one in the HIP model. The N substrate supplying process may however differ from the microbial process of N_2O production and can respond differently to wetting. Besides autotrophic nitrifiers, heterotrophic denitrifiers are also able to use NO_2^- produced via autotrophic nitrification as a substrate for N_2O production (Liu *et al* 2013), which could also explain continued emissions of N_2O_a at high WFPS. High WFPS and therefore reduced soil gas diffusivity is also likely to have delayed N_2O_a 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_2O_a production has been debated (Bakken and Frostegård 2017) as methodological constraints hinder accurate N_2O partitioning (Prosser *et al* 2020). Analogue to biogeochemical models, the source partitioning model used in the study here summarises these processes under N_2O_a 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_a in response to wetting.

The fraction RN_2O_h did not follow a common pattern across soils, reflecting vastly different N_2O and NO_3- production via heterotrophic nitrification. Production of N_2O 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_2O_h and soil pH in the study presented here. The highest emissions of N_2O_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_2O_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 adaption of the soil microbial community to soil chemical properties and N fertilisation (Zhang *et al* 2018a) may explain the differences in partitioning of N_2O production between heterotrophic and autotrophic nitrification with important implication for practical N_2O abatement strategies: Nitrification inhibitors such as 3,4-dimethylpyrazole phosphate (DMPP) inhibit the activity of the ammonia monooxygenase (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_2O production via this pathway. The contribution of heterotrophic nitrification to N_2O 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_2O formation in intensively managed pasture soils.

Fractions of N_2O emitted from autotrophic and heterotrophic nitrification differed in their response to wetting. However, the fraction of overall nitrification emitted as N_2O 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_2O production. The increase of RN_2O_n however suggests denitrification of nitrified N as the process of N_2O production, driven by the rapid depletion of soil O_2. Our findings are consistent with the response of RN_2O_n 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_n independent of soil WFPS is however likely to underestimate N_2O derived from nitrification when simulating large rainfall events.

4.3. The HIP

The response of N_2O production demonstrates an exponential increase of N_2O emissions with the amount of wetting, dominated by denitrification across pasture soils. The immediate reduction of N_2O 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_2O: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_2O production, which appears to be less sensitive to increasing amounts of wetting than autotrophic nitrification. Losses of N_2O_d and N_2O_h suggest that build up and sudden release of C and N substrate upon wetting stimulates N_2O production via denitrification and via the oxidation of organic N. Continuous N_2O 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 N_2O. This is shown by the exponential increase of RN_2O_n with increasing soil water content, suggesting denitrification of nitrified NO_2^- causing N_2O losses from nitrification mediated pathways at high soil water content. The consideration of the NO_2^- pool as a central N pool in models such as in LDNDC may therefore help to accurately simulate N_2O production via nitrification mediated pathways, and further research tracing ^{15}N in the NO_2^- pool is needed to deliver experimental evidence and validation data for this approach. Our findings demonstrate that the proportion of nitrified N lost as N_2O (RN_2O_n) 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 N_2O_n production and corroborate the exponential response of RN_2O_n in models such as SWAT and NOE. Importantly, the RN_2O response curves established in our study combine physical, chemical, and biological effects of wetting on N_2O 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 N_2O production suggests an opportunity to improve models by adding further pathways of N_2O production. The resulting complexity however needs to be weighed against potential benefits, ensuring accurate N_2O 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.

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