Amplitude and Frequency of Wetting and Drying Cycles Drive N₂ and N₂O Emissions From a Subtropical Pasture

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Abstract

Changes in amplitude and frequency of wetting and drying cycles in pasture systems due to climatic variation and irrigation management are likely to affect magnitude and partitioning of N_2 and N_2O emissions. This study investigated the effects of irrigation frequency on N_2 and N_2O emissions from an intensively managed pasture in the subtropics. Irrigation volumes were estimated to replace evapotranspiration and were applied either once (Low-Frequency) or split into four applications (High-Frequency). To test for legacy effects, a large rainfall event was simulated at the end of the experiment. Over 15 days, $7.9 \pm 2.7 \text{ kg } \text{N}_2 + \text{N}_2\text{O} - \text{N } \text{ha}^{-1}$ was emitted on average regardless of irrigation frequency, with N_2O accounting for 25% of overall $\text{N}_2 + \text{N}_2\text{O}$. Repeated, small amounts of irrigation produced an equal amount of $\text{N}_2 + \text{N}_2\text{O}$ losses as a single, large irrigation event. The increase of N_2O emissions after the large rainfall event was smaller in the High-Frequency treatment, shifting the $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ ratio towards N_2 . Our findings suggest that reduced soil-gas diffusivity predominantly drives N_2O and N_2 emissions following large wetting events, while microbial O_2 consumption largely drives N_2O and N_2 emissions after small, repeated wetting events in high N turnover pasture soils. The abundance of marker genes for N cycling did not differ between treatments, indicating that N and C availability, not gene abundance determined magnitude and $\text{N}_2\text{O}:\text{N}_2$ partitioning after rainfall. The observed legacy effect suggests that increased irrigation frequency can reduce the environmental impact (N_2O), but not overall magnitude of N_2O and N_2 emissions from intensively managed pastures.

1. Introduction

Wetting and drying cycles lead to rapid changes in soil moisture in pasture soils, triggering pulses of nitrous oxide (N_2O) and, if the microbial process of denitrification goes to completion, dinitrogen (N_2) emissions. Emissions of N_2 and N_2O are governed by complex feedbacks between nitrogen (N) and carbon (C) substrate availability (Azam et al. 2002; Giles et al. 2012), temperature (Blagodatskaya et al. 2014; Stres et al. 2008), soil pH (Baggs et al. 2010; Čuhel and Šimek 2011), soil oxygen (O_2) status (Rohe et al. 2021) and the physiological response of the soil microbial community to these factors (Kuypers et al. 2018). Soil moisture exerts an overarching control, as it determines substrate diffusion, but also diffusion of gases into and within the soil matrix (Blagodatsky and Smith 2012), and thus the production and the movement of N_2O and N_2 in the soil, defining temporal variability, overall magnitude, and $\text{N}_2\text{O}:\text{N}_2$ partitioning of resulting N_2O and N_2 surface emissions. The $\text{N}_2\text{O}:\text{N}_2$ ratio defines the environmental impact of these emissions, since N_2O is a potent greenhouse gas, and the single most ozone depleting substance in the stratosphere (Ravishankara et al. 2009). Shifts in amplitude and frequency of wetting and drying cycles under current climate change scenarios, but also changes in irrigation schemes are likely to cause shifts in microbial function (Evans and Wallenstein 2012), in microbial activity (Banerjee et al. 2016) and ultimately in production of N_2O in pasture soils. The general lack of in-situ-measurements of N_2 however hinders accurate predictions how the role of pasture soils as sources and sinks of N_2O will change under the predicted changes of wetting and drying cycles.

Enzyme activity related to N cycling is tightly linked to O₂ availability in soils: The rate limiting step of nitrification, the oxidation of the NH₃ to hydroxylamine by the ammonia mono-oxygenase (AMO) requires O₂ and is therefore inhibited under anaerobic conditions. Classic heterotrophic denitrifiers (Braker et al. 2012), but also other organisms capable of denitrification (Wrage-Mönnig et al. 2018) switch to NO₃⁻ or nitrite (NO₂⁻) as electron acceptors if O₂ becomes limiting, producing variable amounts of nitric oxide (NO), N₂O and N₂. The activities of the responsible enzymes, i.e. the NO₂⁻ reductase (NIRS), the NO reductase (NORB) and the N₂O reductase (NOS), are negatively correlated to O₂ availability, with NOS being the most sensitive to O₂ inhibition (Morley et al. 2008).

Relative soil gas diffusivity (D_p/D_o) describes potential O₂ diffusion through and into the soil, accounting for the interaction between bulk density, pore size distribution and soil water content (Moldrup et al. 2013). In contrast to soil water filled pore space (WFPS), D_p/D_o enables comparisons between soils with differing bulk densities (Farquharson and Baldock 2008). The onset of anaerobic conditions in the soil has been reported for $D_p/D_o < 0.02$ (Stepniewski 1981), while decreasing N₂O emissions due to increased reduction to N₂ were observed at $D_p/D_o < 0.006$ (Balaine et al. 2016). The negative correlation between D_p/D_o and N₂O (Clough et al. 2020) suggests that wetting and drying cycles that maximise D_p/D_o will minimise the formation of anaerobiosis in soils, limiting N₂O and N₂ emissions. This hypothesis has been supported by irrigation studies in cropping (Jamali et al. 2015) and pasture (Rousset et al. 2021) systems, where the split application of irrigation increased soil aeration and thus reduced N₂O emissions. However, soil O₂ concentration is defined by the balance of supply and consumption (Rohe et al. 2021), and N₂ and N₂O emissions well above the postulated D_p/D_o threshold have been attributed to increased microbial O₂ consumption driven by easily available C in pasture soils (Friedl et al. 2021; Petersen et al. 2013). Both O₂ consumption and reduced D_p/D_o will ultimately determine magnitude and partitioning of N₂O and N₂ emissions, yet their significance is likely to shift as the amplitude and frequency of wetting and drying cycles changes.

This study is part of a trial on an intensively managed dairy pasture, where effects of increased irrigation frequency, that is more frequent and small irrigation events versus large and infrequent events, on N cycling and loss were investigated. Previous work at the site (Mumford et al. 2019) demonstrated no effects of increased irrigation frequency on pasture yield or plant N uptake, but a reduction of N₂O emissions. This reduction was not directly linked to irrigation events but to a treatment legacy observed after intense rainfall.

Building on these results, the aim of the study presented here was a) to establish the response of both N₂O and N₂ emissions to increased irrigation frequency and b) to further investigate the previous observed treatment legacy on N₂O emissions, linking in-situ measurements of N₂O and N₂ from an intensively managed dairy pasture to D_p/D_o , C and N substrate availability and the abundance of functional marker genes for N cycling. We hypothesised that a) increased irrigation frequency would reduce overall N₂ and N₂O losses and shift the N₂O/(N₂O+N₂) ratio towards N₂, and b) that the treatment

legacy after large rainfall was linked to reduced NO_3^- availability and a subsequent decrease in the $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ ratio.

2. Material And Methods

The study was conducted on a commercial dairy farm in Casino, New South Wales (28.865°E, 152.874°S). Site characteristics including soil physical and chemical parameters are shown in Table 1. The climate at the site is humid subtropical with summer dominated rainfall. Rainfall variation at the site can be extremely large with monthly totals in excess of 400 mm common. Mean annual precipitation is 1037 mm, and average daily temperature ranges from 18°C to 30°C in summer and 6.6°C to 22°C during winter. The soil at the site is a black Vertisol (Isbell 2016), which corresponds to a Pellic Vertisol (IUSS 2015), with clay content increasing from 44% in the topsoil to 50% at depth. The average farm stocking rate is 5 heads of cattle ha^{-1} , supported by average fertiliser inputs of 340 kg N ha^{-1} year^{-1} . On average, 210 head of cattle graze an area of 1.2 ha^{-1} over 12 hours every 14-21 days (De Rosa et al. 2020). Fields are usually grazed and fertilised on a two-to-three-week cycle from the beginning of May until mid-November, with irrigation applied after fertilisation. Fertiliser N rates range from 1-2 kg N ha^{-1} day^{-1} applied as urea (Mumford et al. 2019). The summer dominant Kikuyu pasture (*Pennisetum clandestinum*) is usually mulched at the end of April and oversown with annual ryegrass (*Lolium italicum*).

Table 1

Selected site and soil characteristics for the intensively managed pasture site under dairy production in Casino, NSW, in subtropical Australia.

Parameter		
Coordinates		
Coordinates	152.99 °E; 28.80 °S	
Mean annual rainfall	1107 mm	
Height above sea level (m)	47	
Principle pasture cover (summer)	<i>Pennisetum clandestinum</i>	
Principle pasture cover (winter)	<i>Lolium perenne</i>	
Soil type (ASC)	Vertosol	
Soil type (FAO)	Pellic Vertisol	
Specific soil characteristics	0–30 cm	30-70 cm
Texture class	Medium clay	Heavy clay
Clay (%)	44	55
Silt (%)	27	25
Sand (%)	29	20
Bulk density (g cm ⁻³)	1.22	1.30
pH	6.6	6.7
Electrical conductivity (us cm ⁻¹)	51.7	49.8
Total nitrogen (%)	0.22	0.11
C: N ratio, soil	11.31	12.27
Total organic carbon (%)	2.49	1.35

2.1 Experimental setup

The study was conducted as part of a trial investigating the effects of wetting and drying cycles on N cycling in intensively managed pastures (Mumford et al. 2019). Wetting and drying cycles were simulated using a remote-controlled irrigation system replacing the estimated evapotranspiration (ET) rate by applying the respective amount of irrigation at once (Low frequency - LF) or split into 4 irrigation events (High frequency - HF) over a grazing cycle (Figure 1). Evapotranspiration was estimated based on climate

data from the three previous years and adjusted to crop ET in mm day^{-1} using the respective crop coefficient for ryegrass (Allen et al. 1998). Irrigation was applied with sprinklers at a rate of $0.32 \text{ mm minute}^{-1}$. The LF and HF treatments with four replicate plots of 25 m^2 were established in a randomised plot design with a 1 m buffer between plots in June 2017 after ryegrass establishment (Figure S1), matching the treatments allocation from previous studies. The treatment history is shown in Table S1.

Grazing was simulated by mowing the plots to 5 cm and clippings were removed, protecting monitoring equipment in place. Urea N fertiliser was applied after grazing, followed by an irrigation event (HF or LF) (Figure 1). In the beginning of the ryegrass season, rainfall exclusion shelters ($3.6 * 1.5 \text{ m}$) were established on the plots and opened for each irrigation event. Two steel bases (i.e. microplots) were installed under each shelter 3 weeks before the start of the experiment: A $0.5 \text{ by } 0.5 \text{ m}$ base for soil sampling; and one $0.18 \text{ m by } 0.18 \text{ m}$ base for N_2 and N_2O measurements, all remaining *in-situ* for the length of the experiment. All plots were mowed and fertilised with urea at a rate of 32 kg N ha^{-1} on 31 October 2017. Fertiliser N was applied as a urea solution equivalent to 2 mm irrigation a) at ^{15}N natural abundance on the soil sampling microplot, and b) at 98% ^{15}N atom excess on the $\text{N}_2 + \text{N}_2\text{O}$ micro plot, and c) the rest of the plot received granular urea at the same rate. The HF treatment received 21.4 mm on day 0, 5 and 8 after fertilisation, while the LF treatment received 86.6 mm after fertilisation. To test for legacy effects of irrigation frequency on N_2O and N_2 emissions, a rainfall event of 100 mm was simulated across treatments saturating to top 10 cm of the soil. This was done ten days after fertilisation, when soil WFPS was similar in both treatments (Figure 1, Figure 2) to exclude effects of antecedent soil moisture on N_2O emissions. The first 10 days are referred to as Phase 1, followed by the rainfall event (Phase 2) and the days after rainfall (Phase 3) (Figure 2).

2.1 Gas sampling and analysis

Manual gas samples

The static closed chamber method was used to measure N_2 , N_2O and CO_2 emissions. Manual gas samples were taken at day 1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13 and 15 days after fertilisation, with samples taken between 9 am and 12 am. Polyethylene chambers with a headspace height of 20 cm for the N_2 micro plots were placed on the steel frames, and headspace gas samples (20 ml) were taken by connecting a syringe to a 2-way luer-lock tap installed in the lid of the chamber 0, 60 and 180 minutes after chamber closure. Gas samples were injected into a pre-evacuated 12 ml glass vial with a double wadded Teflon/silicon septa cap (Labco, UK). Temperature inside the chambers was recorded with a temperature logger (HOBO onset UA-002-64).

Headspace gas samples were analysed for N_2O and CO_2 by gas chromatography (GC) (Shimadzu GC-2014), and for the isotopologues of N_2 ($^{15}\text{N}^{14}\text{N}$, $^{15}\text{N}^{15}\text{N}$) and N_2O ($[^{14}\text{N}^{15}\text{N}^{16}\text{O} + ^{15}\text{N}^{14}\text{N}^{16}\text{O}]$ and $^{15}\text{N}^{15}\text{N}^{16}\text{O}$) using an automated isotope ratio mass spectrometer (IRMS) coupled to a trace gas preparation unit (Sercon Limited, 20-20, UK).

2.2 Aboveground biomass sampling and analysis

At the end of the experiment, 2 biomass cuts at grazing height (>5 cm) from random positions under the rain shelter were bulked from each plot using a 0.5m × 0.5 m metal quadrat. Aboveground biomass was harvested at grazing height (>5 cm) from 3 random plants. Plant material was oven dried at 60°C, ground with a planetary cylinder mill, and analysed for N content using a LECO TruMac CNS analyser (MI, USA).

2.3 Soil sampling

Destructive soil samples were taken from the soil sampling microplots one day prior, and 2, 4, 6, 10, 12, 13, and 15 days after fertilisation using a soil corer with an (inner diameter 17mm). For soil mineral N, dissolved organic N (DON) and C (DOC) analysis, 3 bulk samples (0-10 cm) from the topsoil were taken from each microplot, bulked together, stored at 5°C at the site. For DNA extraction, 3 bulk samples (0-10 cm) were homogenized and separated from the root biomass on site. Aliquots of 250 mg were directly suspended in extraction buffer and stored at 5°C. Both bulk soil samples and soil in extraction buffer were transported cooled to the laboratory within a day of sampling.

2.4 Soil mineral N, DON and DOC

Soil samples were extracted with a 2 M KCl solution (1:5 w:v) for 1 h on a rotary shaker and filtered through Whatman no. 42 filter paper. The concentration of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ (measured as a sum parameter of $\text{NO}_3^- + \text{NO}_2^-$) in the KCl extracts was quantified with a GalleryTM Discrete Analyzer (Thermo Fisher Scientific). Soil was extracted for DON and DOC with deionised water (1:5 w:v) for 1 h on a rotary shaker, centrifuged (27,179 g, 30 min, 4°C), filtered through a 0.45 µm membrane filter, and analysed by Shimadzu TOC-TN analyser (Shimadzu Corporation, Kyoto, Japan). Soil DON concentrations were calculated as the difference between total N in the water extract and mineral N concentrations.

2.5 Extraction of DNA and quantitative polymerase chain reaction (qPCR) of functional marker genes

Soil DNA was extracted using the PowerSoil[®] DNA Isolation Kit MoBio (Mobio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Concentration of DNA and quality were determined spectrophotometrically (NanoDrop 2000, ThermoFisher, MA, USA). Primer sets used for the quantification of functional marker genes of nitrification (*amoA AOA* and *amoA AOB*) and denitrification (*nirS* and *nosZ*) are listed in Table S2. The qPCR assay was carried out in a volume of 10 µL, and the assay mixture contained Phusion[™] High-Fidelity DNA Polymerase, 10 µM of each Primer (except for the gene *amoA AOA*, where 20 µM were used) and 1 µL of the template DNA. Each sample was pipetted in triplicates using a robot (Eppendorf epMotion 5075t, Eppendorf AG, Hamburg, Germany) and quantified using the CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Standard curves were constructed as described in Friedl et al. (2020b).

2.6 Auxiliary measurements

Soil water content of the HF and LF treatment was logged in 30-minute intervals using domain reflectance probes (SentekTable 2 EnviroSCAN, South Australia) at 5 cm depth. Raw outputs were calibrated using soil water characteristics determined using intact soil cores from the site on a pressure plate apparatus.

2.7 Calculations and statistical analysis

Emissions of N₂O, CO₂ and N₂

The slope of the linear increase or decrease in N₂O and CO₂ concentration during the chamber closure was used to calculate GHG fluxes, corrected for air temperature, atmospheric pressure, and the ratio of chamber volume to the surface area. The coefficient of determination (R²) for linear regression was used as a quality check and flux rates were discarded if R² was <0.80 for N₂O and <0.95 for CO₂.

The ¹⁵N enrichment of the NO₃⁻ pool undergoing denitrification (a_p N₂ and a_p N₂O) and the fraction of N₂ and N₂O emitted from this pool (f_p) were calculated following the equations given by Spott et al. (2006)

$$f_p = \frac{a_m - a_{bgd}}{a_p - a_{bgd}} \quad (1)$$

where a_{bgd} is the ¹⁵N abundance of the atmospheric background and a_m is the measured ¹⁵N abundance of N₂ and N₂O,

$$a_m = \frac{^{29}R + 2 \cdot ^{30}R}{2 \cdot (1 + ^{29}R + ^{30}R)} \quad (2)$$

and ³⁰x_m is the measured fraction of m/z 30 in N₂:

$$^{30}x_m = \frac{^{29}R + 2 \cdot ^{30}R}{2 \cdot (1 + ^{29}R + ^{30}R)} \quad (3)$$

Assuming that N₂ and N₂O derived from the same NO₃⁻ pool undergoing denitrification, a_p derived from N₂O was used to calculate N₂ fluxes. If only ²⁹R was > the detection limit (DL), f_p was calculated as

$$f_p = \frac{1}{1 - \frac{{}^{29}\text{R}(1 - a_p)^2 - 2a_p(1 - a_p)}{{}^{29}\text{R}(1 - a_{bgd})^2 - 2a_{bgd}(1 - a_{bgd})}} \quad (4)$$

Formula 4 was used to calculate 60% of all N₂ fluxes. The headspace concentrations of N₂O and N₂ were multiplied by the respective f_p values giving N₂ and N₂O produced via denitrification (referred to as N₂ and N₂O_d), with their respective fluxes expressed in g N₂ or N₂O_d -N emitted g⁻¹ soil day⁻¹.

The between batch precision of the IRMS for N₂ based on the standard deviation of atmospheric air samples (n=36) at 95% confidence interval (Friedl et al. 2020a) was 4.4×10^{-7} and 6.61×10^{-7} for ²⁹R and ³⁰R, respectively. The corresponding method detection limit ranged from 34 g N₂-N ha⁻¹ day⁻¹ with a_p assumed at 60 atom % to 303 g N₂-N ha⁻¹ day⁻¹ with a_p assumed at 20 atom %.

Soil gas diffusivity D_p/D_o was calculated for 0 - 10 cm soil depth using the structure dependent, water induced linear reduction model of Moldrup et al. (2013) with the media complexity factor of 2.1 for intact soil.

The effect of irrigation frequency on emissions of N₂ and N₂O, and the abundance of *nosZ* and *nirS* was assessed using linear mixed-effect models (R Package “nlme”) (Pinheiro et al. 2019)

Relative changes of the N₂O/(N₂+N₂O) ratio and substrate availability for denitrification (DOC, NO₃⁻) in response to the rainfall event were calculated as the difference in the plot specific values prior and after rainfall. The effect of irrigation frequency on relative changes of the N₂O/(N₂+N₂O) ratio and substrate availability for denitrification (NO₃⁻, DOC), was analysed by analysis of variance (ANOVA) (P < 0.05). Values in the text, tables and figures represent means ± standard error of the mean.

3. Results

The response of soil water content, soil gas diffusivity (D_p/D_o) and emissions of N₂ and N₂O to different wetting and drying cycles (HF and LF) and the rainfall event is shown in Figure 2.

3.1 Soil water and soil gas diffusivity

Actual ET at the site was 75.7 mm, 13% lower than ET estimated on the average of the previous 3 years (86.6mm). The application of 86.6 mm of irrigation after fertilisation to the LF plots increased soil WFPS >75%, and respective values for D_p/D_o decreased below 0.006 for 1.5 days. Soil D_p/D_o increased thereafter but remained below 0.02 until day 10. On the HF plots, 21.4 mm of irrigation increased soil WFPS from 51 to 58%. Corresponding values for D_p/D_o fell below 0.02 for one day and increased thereafter. Irrigation events on day 4 and 8 lowered D_p/D_o below 0.02 for one and two days, respectively.

The soil water content on day 10 was 53 and 51% WFPS for the HF and the LF, respectively. The simulation of a 100 mm rainfall event at day 10 increased soil WFPS >90%, decreasing D_p/D_o below 0.006 for 1.5 days in both treatments. Soil water content decreased until day 15 and values for D_p/D_o remained below 0.02. Regardless of treatment, WFPS remained above field capacity (54%) except for day 8 after fertilisation in HF.

3.2 Emissions of N_2O and N_2

Fluxes of N_2O on day 1 after fertilisation ranged from 125 to 330 g $N_2O-N\ ha^{-1}day^{-1}$ (Figure 2). Values for average daily N_2O fluxes were higher from LF as compared to HF for the first three days after fertilisation, but differences were not significant. Irrigation on day 4 in HF increased N_2O emissions > 100 g $N_2O-N\ ha^{-1}day^{-1}$, while respective fluxes from LF remained below 50 g $N_2O-N\ ha^{-1}day^{-1}$. The simulated rainfall event led to a pulse of N_2O emissions for a day in both treatments, with average daily fluxes of 743 ± 33 and 1454 ± 692 g $N_2O-N\ ha^{-1}day^{-1}$ for the HF and LF, respectively. The variability between replicates was high, with N_2O fluxes ranging from 250 to 1710 g $N_2O-N\ ha^{-1}day^{-1}$ for HF, and from 472 to 3502 g $N_2O-N\ ha^{-1}day^{-1}$ for LF. Subsequent N_2O fluxes decreased to < 100 g $N_2O-N\ ha^{-1}day^{-1}$.

Emissions of N_2 from LF on the first day after fertilisation were > 400 g $N_2-N\ ha^{-1}day^{-1}$, and exceeded N_2 emissions from the HF by a factor of 2 for the first two days. The irrigation event on day 4 increased N_2 emissions from the HF treatment > 300 g $N_2-N\ ha^{-1}day^{-1}$. The simulated rainfall event led to a sharp increase of N_2 emissions, with 1140 ± 312 and 1737 ± 475 g $N_2-N\ ha^{-1}day^{-1}$ emitted from HF and LF, respectively. Fluxes of N_2 decreased in both treatments until day 13. On day 15, N_2 emissions > 1000 g $N_2-N\ ha^{-1}day^{-1}$ were observed from both treatments.

Regression analysis (Figure 3) showed that the log of daily N_2O-N and N_2-N followed a linear relationship with log D_p/D_o ($R^2 = 0.72$ and $R^2 = 0.43$, respectively; $P < 0.01$) in LF. This relationship was not observed for HF.

Cumulative N_2+N_2O losses from the LF treatment exceeded those from HF for the first 5 days ($P < 0.05$). Values for average cumulative N_2+N_2O emissions were higher from LF as compared to HF after day 10, but the difference was not significant. Over 15 days, 7302 ± 1476 and 8593 ± 1334 g $N_2+N_2O-N\ ha^{-1}$ were emitted from HF and LF, respectively. Emissions of N_2O accounted for 22 and 25% of overall N_2+N_2O losses from HF and LF, respectively. Emissions of N_2O and the $N_2O/(N_2O+N_2)$ ratio did not differ between treatments before the rainfall event. After rainfall, emissions of N_2O increased in both treatments and exhibited high variability within treatments. The relative increase in N_2O from day 10 to 11 was higher in LF, exceeding the increase observed for HF by a factor of 4 ($P < 0.05$). The proportion of N_2+N_2O emissions lost as N_2O increased from day 10 to day 11 from 4 to 37% for LF, and from 19 to 37% for HF. This shift in the $N_2O/(N_2O+N_2)$ ratio was significantly higher in LF as compared to HF ($P < 0.05$).

The enrichment of the NO_3^- pool undergoing denitrification ($a_p \text{ N}_2$ and $a_p \text{ N}_2\text{O}$) is plotted in Figure S2, showing a decrease of $^{15}\text{NO}_3^-$ enrichment over time in both treatments over the time of the experiment. Differences between $a_p \text{ N}_2$ and $a_p \text{ N}_2\text{O}$ were not statistically significant. However, average values for $a_p \text{ N}_2$ were below $a_p \text{ N}_2\text{O}$ on some days in the first half of the experiment.

3.3 Emissions of CO_2

Emissions of CO_2 (Figure S3) were used as an indicator of relative differences in heterotrophic soil respiration between treatments, as different wetting and drying cycles had no effect on pasture yield (Mumford et al. 2019). Fluxes of CO_2 decreased after an initial peak in both treatments. In contrast to LF, CO_2 emissions in HF responded to irrigation events, and were higher than those from LF. Emissions of CO_2 increased after the rainfall event, with no differences between treatments.

3.4 Soil mineral N, DON and DOC

Soil mineral N and DON concentrations did not differ between treatments (Figure 4). Soil NH_4^+ concentrations remained stable over the time of the experiment, ranging from 12 to 19 $\text{mg NH}_4^+\text{-N kg}^{-1}$ soil. Soil NO_3^- concentrations increased after fertilisation and peaked at day 5 in both treatments at 19 to 22 $\text{mg NO}_3^-\text{-N kg}^{-1}$ soil and subsequently decreased to 5 $\text{mg NO}_3^-\text{-N kg}^{-1}$ at the end of the experiment. Soil DON and DOC increased over time and peaked at day 12 of the experiment, decreasing thereafter. Soil DON ranged from 38 to 100 mg DON-N kg^{-1} soil and did not differ between treatments. Soil DOC ranged from 451 to 1245 mg DOC-C kg^{-1} soil. The rainfall event increased the DOC concentration in HF by a factor of 1.4 from day 10 to 12 ($P < 0.05$).

3.5 Aboveground biomass

Aboveground biomass (>5 cm) yield was low at the end of the ryegrass season and showed no treatment effect, averaging $0.75 \pm 0.27 \text{ t DM ha}^{-1}$ across treatments. With an N content of $4.31 \pm 0.12\%$, N yield was $32.51 \pm 12.17 \text{ kg N ha}^{-1}$.

3.6 Abundance of N cycling functional marker genes

The abundance of ammonia oxidising archaea (AOA) and bacteria (AOB) was quantified determining archaeal and bacterial *amoA* gene copy numbers. Archaeal gene copy numbers fluctuated between 1.7×10^7 to $6.8 \times 10^7 \text{ g}^{-1}$ dry soil and exceeded bacterial *amoA* gene copy numbers by around an order of magnitude (Figure 5). The abundance of *nirS* as a proxy for functional genes involved in NO_2^- reduction ranged from 3.5×10^6 to $1.6 \times 10^6 \text{ g}^{-1}$ dry soil. Gene copy numbers of *nosZ* ranged from 5.0×10^7 to $1.7 \times 10^7 \text{ g}^{-1}$ dry soil, around an order of magnitude below the *nirS* copy numbers. The long-term simulation of different wetting and drying cycles had no significant effect on the abundance of functional marker genes quantified prior to fertilisation. The linear mixed effect models did not show a significant effect of

sampling date on the abundance of the investigated functional marker genes during the experiment, and thus no response to different wetting and drying cycles (HF and LF) and the rainfall event.

4. Discussion

4.1 Effect of irrigation frequencies on N_2 and N_2O emissions

Emissions of N_2 and N_2O responded to wetting and drying and were dominated by N_2 , exceeding N_2O emissions by a factor of 4 over the time of the experiment. Peak N_2 and N_2O fluxes were consistent with spikes in soil WFPS causing declines in soil gas diffusivity (D_p/D_o), suggesting increased anaerobicity promoting denitrification. The application of ^{15}N labelled urea hinders N_2O source partitioning (Arah 1997) between nitrification and denitrification, as the NH_4^+ pool cannot be assumed to be at natural ^{15}N abundance. The relationship between soil water content and N_2O emissions however suggests denitrification as the main pathway of N_2O production; an assumption which is supported by results from an incubation study with the same pasture soil (Friedl et al. 2021), demonstrating denitrification as the main source of N_2O at WFPS $\geq 40\%$.

Differences in wetting and drying were reflected in the temporal response of D_p/D_o (Figure 2): As expected, D_p/D_o was higher in the HF treatment in phase 1 of the experiment. In the LF treatment, D_p/D_o fell below the proposed threshold of 0.006 (Balaine et al. 2016) after the initial irrigation of 84 mm, while D_p/D_o remained well above 0.006 in the HF treatment, and dropped only below 0.02 (Stepniewski 1981) for short periods of time following repeated irrigation events of 21 mm. The comparison between treatments implies increased formation of anaerobic microsites in the LF treatment and therefore larger losses of N_2 and N_2O in phase 1 of the experiment, consistent with the reported reduction of N_2O from urine patches when maximising D_p/D_o (Rousset et al. 2021). However, cumulative N_2 and N_2O emissions after two irrigation events of 21 mm were as high as those observed after a single irrigation event of 84 mm, showing that the frequency of wetting events, and not just the absolute amount of soil water was driving the magnitude of N_2 and N_2O emissions. Pooling N_2 and N_2O emissions across D_p/D_o (Figure 3) further highlights differences between treatments: The loglinear relationship between daily N_2O -N and N_2 -N and D_p/D_o shows an exponential increase of both N_2O and N_2 with decreasing D_p/D_o when emissions are triggered by single and large rainfall/irrigation events. This relationship does not hold in the HF treatment, indicating that the physical effects of increased D_p/D_o are superseded by other processes stimulating emissions of N_2 and N_2O . Previously collected pasture biomass data over multiple cuts demonstrated no response of biomass yield to different wetting and drying cycles (Mumford et al. 2019). With no differences in pasture growth, the positive response of CO_2 emissions in the HF treatment to irrigation can be interpreted as an increase in microbial activity (Samad et al. 2016), inducing increased O_2 consumption (Meyer et al. 2010; Rohe et al. 2021), which promotes N_2 and N_2O emissions via

denitrifying pathways. This assumption is consistent with increased C and N mineralisation in response to wetting and drying (Borken and Matzner 2009) and the subsequent increase in C and N and availability which promotes microbial activity. Furthermore, the increase of labile C availability (DOC) in response to wetting and drying cycles has been shown to be strongest in grassland soils compared to cropping and forest soils (Dong et al. 2021). Reductions of N₂O emissions by increased irrigation frequency reported from cropping soils (Jamali et al. 2015) may therefore not be transferrable to pasture soils characterised by high organic C and N content. Our results suggest that the increase in microbial activity and related O₂ consumption in response to small and repeated wetting events can offset the effects of increased D_P/D_O on denitrification and highlight the limitation of D_P/D_O as a sole predictor for N₂O and N₂ emissions.

4.2 Legacy effect of wetting and drying cycles on N₂:N₂O partitioning

The simulated rainfall event increased N₂O emissions in both treatments, exhibiting high variability within treatments. However, the relative increase of N₂O after rainfall was more pronounced in the LF treatment, also showing an increased shift of the N₂O/(N₂O+N₂) towards N₂O. Soil moisture in both treatments prior rainfall was comparable, meaning that antecedent soil moisture (Bergstermann et al. 2011) and thus the immediate effect of wetting did not cause the increased shift of the N₂O/(N₂O+N₂) ratio towards N₂O in the LF treatment. Long term rainfall manipulations (Evans and Wallenstein 2012) and drought (Canarini et al. 2021) studies have shown that historical soil water content can alter the response of soil microbes to rainfall events. Previously observed reductions of N₂O in the HF treatment during periods with rainfall only (Mumford et al. 2019) have been attributed to persistent legacy effects of wetting and drying cycles on ammonia oxidisers (Fierer and Schimel 2002), and NO₃⁻ substrate availability and its effect on the N₂O/(N₂O+N₂) ratio (Friedl et al. 2020b; Senbayram et al. 2019). Gene copy numbers of archaeal and bacterial *amoA* did not show any treatment effects on the abundance of nitrifiers prior the experiment, nor did they respond to wetting and drying cycles during the experiment (Figure 5). Even though the abundance of marker genes is not necessarily indicative for transcription, and further for enzyme activity, the data provides no indication that the postulated legacy effect is explained by the abundance of nitrifiers, or by differences in NO₃⁻ supply and/or NO₃⁻ availability. Similar to nitrification, the abundance of *nosZ* carrying denitrifiers (clade I) showed no significant treatment response, and differences between treatments regarding the increase of N₂O after the rainfall event were not reflected in *nosZ* abundance. It is noteworthy that the sole quantification of *nosZ* clade I provides only information on this particular clade, and the inclusion of recently developed primers covering a broader range of taxa for *nosZ* clade I (Zhang et al. 2021), and *nosZ* clade II (Hallin et al. 2018) may produce better correlations with N₂O emissions (Xu et al. 2020) and their further reduction to N₂. The abundance of *nosZ* carrying organisms as observed in the study presented here suggests that differences in N₂O fluxes and the increased reduction to N₂ is not explained by differences in abundance of *nosZ* carrying organisms, but by factors driving their response to large rainfall events. In contrast to N substrate availability, DOC availability differed between treatments and was higher in HF than LF after the rainfall event. Carbon is unlikely

limiting for heterotrophic N_2O producing and reducing organisms in this pasture soil with more than 2.5% organic C content. However, increased C availability has been linked to increased reduction of N_2O to N_2 (Friedl et al. 2021; Putz et al. 2018), which may explain the observed differences in N_2O and N_2 : N_2O partitioning. Our findings suggest an important link between C mineralisation and the observed effect of historical wetting and drying cycles on N_2O production, and indicate that under field conditions, C and N substrate availability (Banerjee et al. 2016; Németh et al. 2014), not gene abundance defines the response of the N cycling microbial communities and therefore the response of N_2O production to rainfall.

4.3 Decoupling of N_2O and N_2 emissions

The temporal pattern of gas fluxes in phase 3 of the experiment shows a decoupling of N_2O and N_2 emissions, with N_2 fluxes increasing to $> 1000 \text{ g N}_2\text{-N ha}^{-1} \text{ day}$, and N_2O emissions decreasing to values below $20 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}$. Delayed emissions of N_2 after rainfall events have been attributed to reduced D_p/D_o and subsequent entrapment of both N_2O and N_2 in the soil matrix (Clough et al. 2005). This is consistent with the high N_2 fluxes observed in this study, implying the release of previously produced N_2 as D_p/D_o increases after the rainfall event. This assumption is further supported by the low N_2O emissions, as retention of N_2O in the soil matrix favours complete denitrification to N_2 (Hansen et al. 2014). Soil layers below 0.5 m depth in these heavy clay Vertisols under pasture rarely dry out when irrigated. Applied N and moisture can accumulate in these layers after rainfall, providing conditions favouring N_2O and N_2 production. The delayed peak of N_2 fluxes observed in this study may therefore be attributed to the overlapping effects of gas entrapment and subsoil denitrification after rainfall, and future research should account for their effect on magnitude and temporal variability of N_2 and N_2O emissions from intensively managed pastures.

5. Conclusions

This study delivers critical data on how both N_2O and N_2 emissions respond to differences in wetting and drying cycles when water availability remains optimal for pasture growth. Repeated, small amounts of irrigation produced the same amount of N_2 and N_2O as a single and large irrigation event, highlighting that frequency, not only the amplitude of wetting and drying cycles drives the magnitude of N_2 and N_2O emissions from pasture soils. This study captured N_2O and N_2 emissions over a single grazing cycle, and further research is needed to expand our findings across a wider range of environmental conditions and to investigate the persistence of effects of historical wetting and drying regimes. Even though mechanisms are likely overlapping, we postulate that physical effects of reduced soil gas diffusivity (D_p/D_o) predominantly drive N_2O and N_2 emissions in response to single and large wetting events. Microbial activity and ensuing O_2 consumption are however likely the main factors driving production of N_2O and N_2 in response to small but repeated wetting events. Our findings highlight the limitation of modelling approaches using only soil water content as a predictor for O_2 availability when forecasting the response of N_2O and N_2 emissions to wetting and drying cycles. The lack response of cumulative N_2 and

N₂O emissions to the irrigation treatments shows limited scope of increased irrigation frequency to reduce N₂ and N₂O triggered by irrigation from pasture soils characterised by high N turnover. However, the observed legacy effect of wetting and drying cycles after rainfall indicates that increased irrigation frequency can reduce N₂O emissions and shift the N₂O/(N₂O+N₂) ratio towards N₂ following large rainfall events, reducing the environmental impact, but not the overall magnitude of N₂O and N₂ emissions from intensively managed pastures.

Declarations

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Conflict of interest

The authors declare no competing interests that are relevant to the content of this article.

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Figures

Figure 1

Outline of the experiment showing grazing, N fertilisation and the application of the high (light blue) and low frequency (dark blue) irrigation treatments and the simulated rainfall event (black) as a percentage of estimated evapotranspiration for 6 cycles of irrigation history and during the time of the experiment. Resulting water filled pore space (WFPS) is shown for the experimental period.

Figure 2

Daily emissions of N_2O and N_2 , cumulative emissions of $\text{N}_2\text{O}+\text{N}_2$ ($\text{g N ha}^{-1} \text{ day}^{-1}$), soil WFPS, soil gas diffusivity (D_p/D_o) from an intensively managed dairy pasture in response to high frequency (HF) irrigation, low frequency (LF) irrigation and a simulated rainfall event. The continuous line in the WFPS figure shows field capacity. The continuous line in the D_p/D_o graph marks the proposed threshold for the formation of anaerobic sites in the soil matrix (Stepniowski, 1981) and the dashed line shows the proposed threshold for maximum N_2O production (Balaine et al., 2016).

Figure 3

Relationship of pooled daily N_2 and N_2O fluxes and soil gas diffusivity from an intensively managed dairy pasture in response to high frequency (HF) and low frequency (LF) irrigation shown as the regressions of their respective log values.

Figure 4

Soil mineral N (NH_4^+ and NO_3^-), dissolved organic N (DON) and C (DOC) concentrations from an intensively managed dairy pasture in response to high frequency (HF) irrigation, low frequency (LF) irrigation and a simulated rainfall event.

Figure 5

Gene copy numbers over time for functional genes of functional marker genes for nitrification (bacterial *amoA* and archaeal *amoA*) and denitrification (*nirS* and *nosZ*) from an intensively managed dairy pasture in response to high frequency (HF) irrigation, low frequency (LF) irrigation and a simulated rainfall event.

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