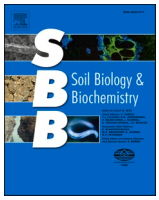
Soil Biology and Biochemistry 168 (2022) 108606

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio



The impact of drought length and intensity on N cycling gene abundance, transcription and the size of an N_2O hot moment from a temperate grassland soil

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ARTICLE INFO

Keywords:
Soil moisture
Nitrous oxide
Dry wet cycles
Legacy
Water filled pore space

ABSTRACT

This study aimed to investigate the relationship between drought length, drought intensity and the size of the N_2O hot moment. It selected two treatments to deduce the main nitrogen cycling process producing N_2O (increasing WFPS from 40% to 90%, and from 70% to 90%), by destructively sampling soil cores to analyse gene abundance, transcription, and changes in soil chemistry (TON, NH_4^+, DOC). Five other drought and rewetting treatments on packed soil cores were selected to create the drought curves described in Barrat et al. (2020): these included increases of WFPS from 40% to 90%, 50%–90%, 60%–90%, 70%–90%, and 30%–60%. For each treatment, drought lengths were imposed from 0 to 30 days. A quadratic linear regression was fitted to the cumulative emissions data. This model explained a significant proportion of the total variation in the data ($R^2 = 0.72$, $p \leq 0.001$). All treatments had an increase in daily N_2O emissions post wetting typical of a hot moment apart from the 30%–60% WFPS treatment. In terms of drought intensity, the 40%–90% WFPS was significantly larger than rest, probably due to a relatively larger change in water potential compared to the other treatments. The response to drought length followed a quadratic curve with a downward linear trend, with the largest emissions observed between 10 and 15 days of drought, and the smallest at 0 and 30 days. We suggest a 2-stage dormancy strategy to explain this, where microbes under dry conditions store osmolytes which are catabolised upon rewetting, however at prolonged negative water potentials this strategy is no longer effective, and so they enter a deeper state of dormancy where they can no longer rapidly respond to the changing water potential. Given the delayed response after rewetting, and the inverted U shaped curve in terms of drought length, it seems likely that the majority of emissions are of biological origin. The soil's chemistry data suggested that NH_4^+ was a key factor controlling the emission flux, but the transcriptional and genomic data were inconclusive. This study therefore suggests that future experiments should focus changes in osmolyte accumulation and catabolism as the key explanation for N_2O hot moments, rather than changes in genomic and transcriptomic data or soil substrates, which do not always correlate with emissions.

1. Introduction

Nitrous oxide (N_2O) emissions can be produced by a rapid and large increase in a soil's water content, resulting in emission events that are many times higher than background levels, these emission events are defined as hot moments (Mcclain et al., 2003, Bergstermann et al., 2011; Harrison-Kirk et al., 2013; Leitner et al., 2017). Because N_2O is a potent greenhouse gas, understanding the dynamics of hot moments is important particularly in determining how changing weather patterns will affect soil N_2O emissions. Dodd et al. (2021) demonstrated that there has been a significant increase in the number of extreme weather events in the UK over the last 28 years, including compound events where drought has been followed by large amounts of rainfall. Moreover, it is predicted that the frequency of drought and large amounts of rainfall is going to increase in the UK due to climate change (Burke et al., 2010; Pendergrass and Knutti, 2018). While it is still unknown how this

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https://doi.org/10.1016/j.soilbio.2022.108606
Received 6 September 2021; Received in revised form 9 February 2022; Accepted 12 February 2022
Available online 26 February 2022

will impact soil emissions, one of the first steps is to determine how the relationship between the length of drought, the degree of rewetting, and the degree of drought before rewetting effects the size of the hot moment.

A recent meta-analysis has made several claims regarding the interaction between the size of the hot moment and the drought and rewetting intensity (Barrat et al., 2020). Using secondary data, this study concluded that the lower the water-filled pore space (WFPS) was before rewetting, and the higher the WFPS was after rewetting, the more likely there would be a larger hot moment due to higher peak emissions and a longer duration. However, it was noted that the studies from which the data for the meta-analysis were extracted lacked consistent methods and a standardized approach, and that no study had investigated how the length of drought impacted N_2O emissions after rewetting.

Therefore this incubation study aimed to test the conclusions of Barrat et al. (2020) while using the studies suggestions for experimental design by using a consistent core size with the same bulk density, and with measurements after the soil is rewetted for at least 4 days. It also aimed to produce the drought impact curves suggested by Barrat et al. (2020) that show the relationship between drought length and the size of the hot moment (i.e. the magnitude of the flux), and how this changes with the drought intensity in terms of the starting WFPS.

In addition, there is still uncertainty regarding the main nitrogen (N) cycling processes that are dominant post wetting. While it seems probable that anaerobic denitrification is the main process, considering the low oxygen content (Baggs, 2011), the classical assumption of reduction from nitrate (NO_3^-) has been challenged by new research into nitrifier denitrification (Wrage-Mönnig et al., 2018), chemodenitrification (Liu et al., 2018), and the possibility of antecedent conditions affecting N_2O reductase (Bergstermann et al., 2011). Unfortunately the lack of studies that control for antecedent conditions and then measure changes in relative abundance of N-cycling transcripts and gene abundance *in-situ*, have inhibited a better mechanistic understanding of the processes involved (Barrat et al., 2020). Barrat et al. (2021) did attempt to discern differences according to functional gene abundance, but no explanatory changes were discovered. There is growing evidence that differences in cell lysis and osmolyte expulsion at the time of rewetting between treatments is unlikely to be the reason for differences in emissions (Barnard et al., 2020; Schimel, 2018; Kakumanu et al., 2013). Instead, the priming effect of the antecedent conditions on the microbial community could be favoring a process that produces more N_2O than a wet control.

This study, using one soil type, is comprised of two experiments. The first experiment aimed to reveal which drought durations and intensities produce the smallest and largest hot moment responses. Following the conclusions of Barrat et al. (2020) we hypothesize that: (H_1), the longer the drought, the larger the hot moment, however this effect will plateau; that (H_2), it will take a minimum number of drying days before the largest hot moment will be observed; and that (H_3), the more intense the drought the greater the hot moment. Also that (H_4), the WFPS after rewetting will have a significant effect on emissions.

The second experiment aimed to reveal which key N cycling processes are dominant at the time of rewetting. As previously work failed to discern differences according to gene abundance and soil chemistry (Barrat et al., 2021), this study aimed to replicate that result while also measuring changes transcript abundance to discern the major N cycling processes. We therefore predicted that (H_5), there would be statistically similar changes in functional nitrogen cycle genes in terms of microbial DNA between treatments and that (H_6), the changes in soil chemistry would not account for differences in the N_2O emissions. However, we predicted (H_7), that changes in transcription abundance would reveal the key N cycling process driving the N_2O emissions.

2. Methods

2.1. Experimental design to create drought impact curves

Five drought and rewetting treatments were selected based on likely values to be measured in summer at the North Wyke Farm Platform (NWFP, https://nwfp.rothamsted.ac.uk/download): 40% WFPS rewetted to 90%, 50% WFPS to 90%, 60% WFPS to 90%, 70% WFPS to 90%, and 30% WFPS to 60%. A high rewetting WFPS was selected for 4 of the treatments, as a previous meta-analysis showed that this was necessary to induce a hot moment (Barrat et al., 2020), and a previous experiment using the same soil showed that 90% WFPS should induce a large hot moment (Barrat et al., 2021). However, this was further tested with the fifth treatment, which was only to 60% WFPS. Each drought and rewetting treatment had 16 different drought lengths (from 0 to 30 days with two-day intervals, e.g. 0 days drought, 2 days drought, 4 days drought … up to 30 days drought) where the soil was kept at its initial WFPS value before being wetted further.

Soil was collected and packed in 80 cores to the same bulk density (5 treatments x 16 drought lengths, see section 2.2), and soil cores incubated in a temperature-controlled room at a constant ~18 °C, where the cores were organized for sampling in a randomized design. The WFPS was adjusted every day to keep it consistent to the specified treatment (see section 2.4). N_2O emissions from each soil core were measured for 14 days after it was rewetted to capture the entire hot moment (see section 2.4). It is worth noting that this experiment was designed to generate data suitable for linear regression analysis capturing the relationship and variation across a range of drought lengths and intensities, therefore replication at each drought length (e.g. 3 replicates at 2 days) was not required as background variation could be captured across the range of drought lengths and treatments (see section 2.6).

2.2. Experimental design for the analysis of N processes

Using the same grassland soil two antecedent moisture treatments were selected that were shown to produce a hot moment from section 2.1.1. One set of 18 soil cores were kept at 40% WFPS for 14 days, which we defined as the pre-dry treatment, and another set of 18 soil cores were kept at 70% WFPS for 14 days, which we defined as the pre-wet treatment, both were then rewetted to 90% WFPS and held there for 7 days. Destructive sampling of the cores for chemical and biological analysis was informed by the daily N_2O emission data (see section 2.5 and 2.6), and at each time point 3 replicates from each treatment were sampled. Soil was collected and prepared and emissions were measured in the same manner as section 2.1.1. A visual summary of both experiments is outlined in Fig. 1.

2.2.1. Soil collection and preparation

Soil was collected from Rothamsted Research's Rowden grassland site at North Wyke, England, at 9 randomly determined points from the 0–10 cm depth using a trowel. The soil is a clayey pelostagnogley also known as a Stagni-vertic Cambisol, and it is mottled throughout with greyish colours, see Table 1 for other characteristics (Avery, 1973). Roots, stones and vegetation were removed from the soil by hand, and it was air dried for 6 days, reaching a moisture level of 28% WFPS, assuming a packing density of 0.8 g cm^{-3} and a particle density of 2.43 g cm^{-3}. Then a mixed pool of soil was created and sieved through a 9 mm sieve. It was stored for 2 days at 4 °C before 9.44 kg of the air dried soil was weighed into 80 polyethylene bags (118 g per bag) representing each core. When the soil was initially put into each bag it was wetted to 30% WFPS. Then each bag was wetted to half its starting WFPS a day before packing the soil cores, and then a day later they were completely wetted to the appropriate initial WFPS when the cores were packed. This was done to stagger the rewetting and prevent an initial hot moment for soil cores starting at a higher WFPS. The 5 treatments with 16 different drought lengths resulted in 80 cores that were packed to a height of 7.6

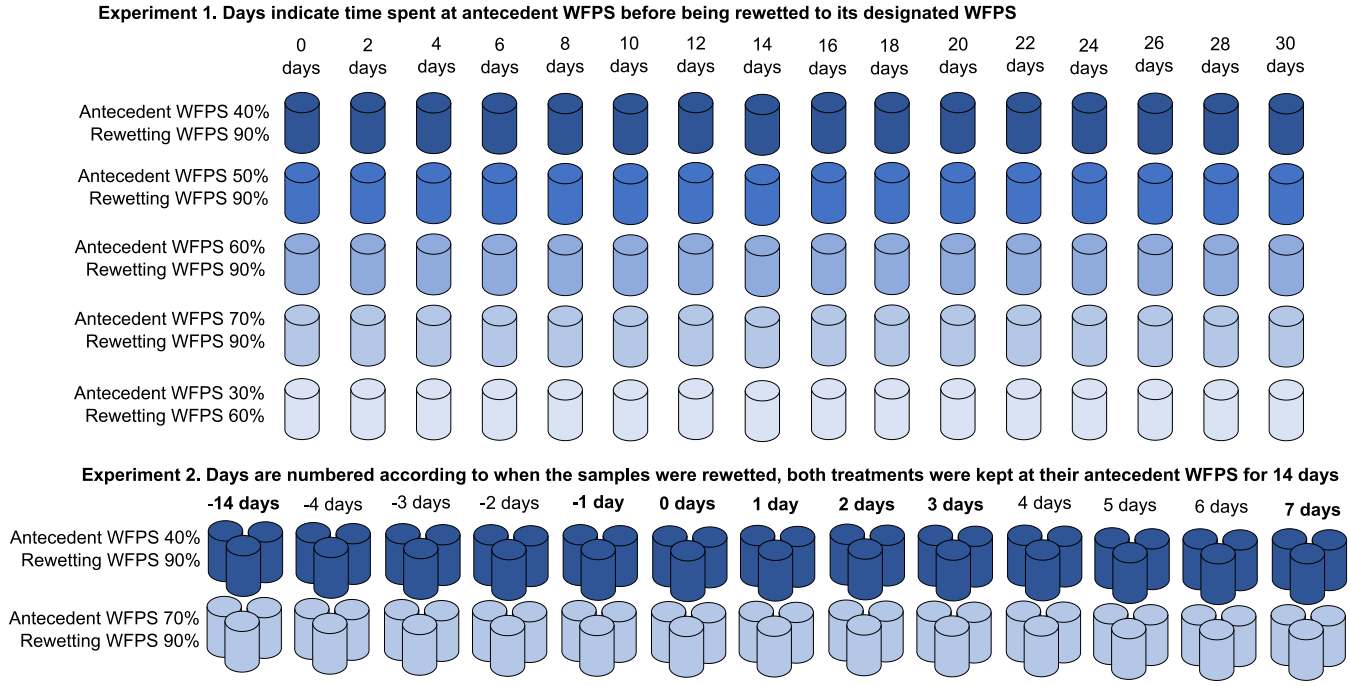

Fig. 1. Visual representation of the two experiments conducted in this study.

Table 1
Summary characteristics of pooled soil used to pack the soil cores for the first experiment. See Fig. 5 for the soil characteristics for the second experiment. Variation is represented using standard error. See section 2.3 for methods.

Vegetation cover	Soil texture	*Soil bulk density in the field g cm^{-3} (n=9)*	Soil pH (n = 5)	Total oxidized nitrogen on a dry soil basis (TON) mg kg^{-1} (n = 5)	*Ammonium (NH_4^+) on a dry soil basis mg kg^{-1} (n=5)*	*Dissolved organic carbon (DOC) on a dry soil basis mg kg^{-1} (n=5)*
Grassland Permanent pasture (*L.perenne*)	Clay	0.77 ± 0.05	6.1 ± 0.1	14.0 ± 0.1	8.2 ± 0.1	146.9 ± 3.1

cm (Greiner Bio-One multipurpose container, 150 ml, metal screw cap, clear, aseptic, item 225,170), at a bulk density of 0.8 g cm^{-3}. The cores were packed by filling and compacting them in thirds, so that the bulk density was consistent throughout the core. The bulk density was selected as a replication of a previous experiment (Barrat et al., 2021).

For the second experiment, soil was collected again 10 days later from the same site, for the packing of cores that were going to be destructively sampled. This pool of soil was air dried for 3 days to reach a moisture level of 40% WFPS, which was determined by taking 3 × 50 g samples each day to determine gravimetric moisture content and assuming a packing density of 0.8 g cm^3 and a particle density of 2.43 g cm^3. It was then passed through a 9 mm sieve and stored for 2 days at 4 °C. Then 4.52 kg of the air dried soil was weighed and divided into 36 polyethylene bags (125.5 g per bag) representing each core. Each bag of soil that represented the 70% WFPS treatment was wetted to 55% WFPS a day before packing the soil cores, and then a day later they were completely wetted to the appropriate initial WFPS when the cores were packed, to stagger the rewetting and prevent an initial hot moment. It is worth noting that the second experiment was not a replication of the first, so drying times and sampling times were different due to the different objectives and treatments.

2.3. Incubation and N_2O measurements

The soil cores were kept in a temperature-controlled room at approximately 18 °C to represent a summer day, and throughout the experiment the cores were weighed daily and the WFPS of each core was adjusted when needed to keep it constant, by adding deionized water assuming weight loss due to evaporation. When measuring emissions, the cores were sealed using modified metal screw caps containing two rubber septa, 3 cm apart, allowing the headspace (49.3 cm^3 at 7.6 cm packing height) to be sampled via PTFE 3.2 mm tubing.

A Picarro cavity ringdown spectrometer G2508 was used to measure the N_2O concentrations. The headspace for each core was sealed for 90 s before sampling, and then the flux was measured for 90 s. The first 10 s of measurement were ignored to allow circulation of the cores' headspace, and the following 80 s of measurement were used to calculate a linear regression from T10 to T90 to generate a flux as outlined by Venterea et al. (2020). This was converted from parts per million (ppm) into a concentration based on the ideal gas law, the chambers dimensions, and the time of measurement (see equation 1).

$$N2O\ flux\ mg\ \mathrm{m2}\ day = \frac{Fppm \times atm}{K \div R \times m} \times \frac{Chv}{Cha} \times 0.76188 \times 1440$$

Equation 1. Used to calculate emissions in terms of grams per meter squared per day. Fppm is the ppm flux, atm is the ambient air pressure, K is the air temperature in kelvin, R is the ideal gas constant in L atm mol^{-1} K^{-1}, m is the moles of N in the N_2O molecule (28), Chv is the chamber volume in m^3 and Cha is the chamber area in m^2, 0.76188 converts the flux from 1 min 20 s to 1 min, multiplying by 1440 converts it to a daily flux.

Measurements were taken twice a day (approximately 2 h apart), and an average from the two samplings was taken for each core creating a daily flux. This was converted into the cumulative emissions over 14 days for each treatment, by adding up all the daily emissions for 14 days post wetting (see supplementary material for a summary of the data).

2.4. Soil chemical analyses

Soil chemical characteristics were determined by destructive sampling the soil cores on days: −14, −1, 0, 1, 2, 3 and 7. Day 0 marks the day of rewetting, measurements were taken after the soil was rewetted on this day. Dissolved organic carbon (DOC), total extractable oxidized nitrogen (TON) and ammonium (NH_4^+), was determined on supernatant following 0.5 M potassium sulphate (K_2SO_4) extractions with 10 g of fresh soil in 50 ml of extractant. Samples were shaken for 30 min and then centrifuged (14,000 rpm, 30 min), the supernatant was removed and frozen until later analysis (Jones and Willett, 2006). Inorganic N concentrations were then determined using an Aquakem 250 discrete photometric analyzer. DOC concentrations were determined using a Shimadzu TOC-L CPN total organic carbon analyzer, the samples were diluted by a 10x dilution factor, to prevent precipitation in the instrument. Gravimetric moisture was determined using 50 g samples in an oven at 105 °C for 24 h. Soil pH was determined using a pH meter with a ratio of 5 g–12 ml of deionized water using a Jenway 3320 pH meter.

2.5. DNA and RNA extractions and determination

DNA was extracted from the same time points as the destructively sampled chemical analysis (days −14, −1, 0, 1, 2, 3 and 7) whilst RNA was extracted from all time points post wetting (days 0, 1, 2, 3 and 7). Each time point had 3 true replicates per treatment, and each true replicate consisted of 2 technical replicates. To test changes in functional gene abundance, 2 sub samples of soil were taken per soil core replicate and frozen in 15 ml falcon tubes (~20 g of soil per tube) using dry ice, and stored at −80 °C. The soil samples were then freeze-dried to further preserve the integrity of the nucleic acid and improve homogenisation of the samples. In summary, DNA was extracted from 84 soil samples, and RNA was extracted from 60 soil samples.

DNA was extracted using and following the instructions in the DNeasy PowerSoil kit (Qiagen) in batches of 24 (and one batch of 12), using 250 mg of freeze-dried soil. 50 μl of autoclaved deionized water was added to each sample at the beginning of each extraction to rehydrate the sample. The concentrations of DNA were measured using a Qubit dsDNA BR Assay Kit (ThermoFisher) and the quality was assessed on a Nanodrop spectrophotometer (ThermoFischer). Samples were then diluted to 20 ng μl^{-1} and stored at −80 °C prior to qPCR analysis.

RNA was extracted using the RNeasy PowerSoil Total RNA kit (Qiagen), in batches of 12, using 2.0 g of freeze-dried soil. Samples were pre-weighed before the day of extraction using a weighing spatula that was heated until it was red hot and then cooled in ethanol. We added 400 μl of autoclaved deionized water to each sample after adding phenol, to rehydrate the sample and improve the extraction success. For acidic soils, we found that modifying step 7 by adding 0.75 ml of SR5 and 0.75 ml of SR3 (instead of 1.5 ml of SR3), dramatically increased the extraction success, and so all extractions included this modified step. The RNA samples were purified using the DNase Max kit (Qiagen), and the concentrations of RNA were measured using a Qubit RNA BR Assay Kit (ThermoFisher) and the quality was assessed on a Nanodrop spectrophotometer (ThermoFischer). Samples were then diluted to 20 ng μl^{-1} and stored at −80 °C prior to qPCR analysis.

For DNA samples, qPCR analyses were performed as described in De Sosa et al. (2018). Briefly, using a 384 plate, each run contained 3 different genes, 24 blanks, 2 no-template blanks per gene, 8 × 3 standards per gene, and 2 positive controls using a standard created from a mix of grassland and arable soil. Specific primers (see Supplementary Table 1) were employed to quantify gene abundance of microbial kingdoms and genes associated with N cycling using the QuantiFast SYBR® Green PCR Kit (Qiagen) and a Biorad CFX384 Touch Real-Time PCR Detection System. DNA copy numbers are represented on a per gram of dry soil basis (cn g^{-1}). Results were standardized assuming 40 ng ul per well.

RT-qPCR analysis on RNA samples were performed using a 384 plate, each run contained 4 different genes, 2 blanks, 2 no-template blanks, 8 × 3 standards per gene, and 2 positive controls using a standard created from a mix of grassland and arable soil. Specific primers (see Supplementary Table 1) were employed to quantify gene abundance of microbial kingdoms and genes associated with N cycling using the RT-QuantiFast SYBR® Green PCR Kit (Qiagen) and a Biorad CFX384 Touch Real-Time PCR Detection System. The list of genes analysed and the qPCR extraction efficiency for each gene is stated in Supplementary Table 1.

2.6. Data treatment and statistics for the drought curves

Genstat 20th edition was used for statistical analysis (Vsn, 2020). The experimental data were transformed to satisfy the normality and homogeneity of variance assumptions of the analysis by square rooting the cumulative emissions for each treatment. Curves were then fitted to the data to estimate the relationship between the drought days (on the X axis), and the cumulative N_2O emissions post wetting (on the Y axis) for each drought intensity. These curves were fitted using linear regression with a quadratic term included to capture the curvature of the relationship (see Figs. 2 and 3). Sequential F tests were used to determine significance ($p \leq 0.05$) of the model terms and therefore how complex the final model needed to be to sufficiently describe the relationships.

A one-way ANOVA was used with the cumulative N_2O emissions data as the Y variate and WFPS as the Treatment term and the number of days of drying as the block term. In order to determine if on average the different WFPS intensities affected N_2O emissions (adjusting for drought lengths). Cumulative emissions for each treatment were calculated by averaging the emissions for each treatment for each day and totaling all the days post wetting.

2.7. Data treatment and statistics for the analysis of N processes

The N_2O experimental data were transformed to satisfy the normality and homogeneity of variance assumptions of the analysis by

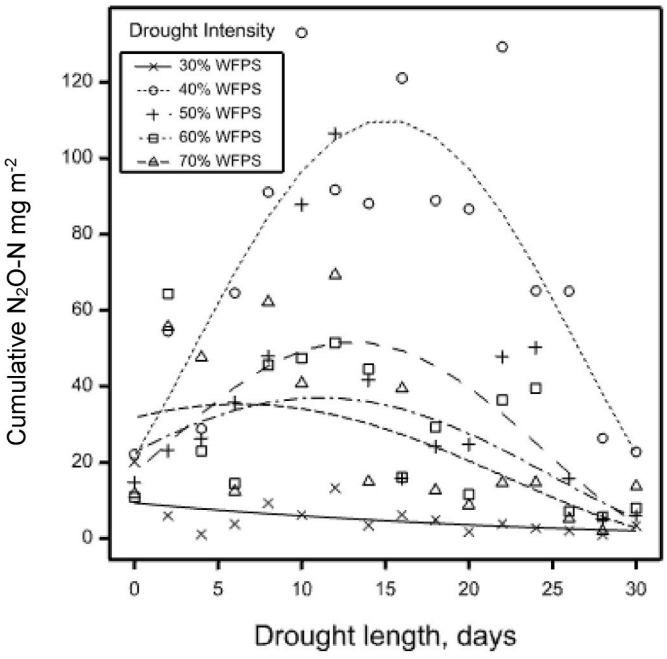

Fig. 2. Scatter plot showing the relationship between the different treatments of drought intensity and drought length, and the response in cumulative N_2O–N emissions in mg m^{-2} for 14 days post wetting. All treatments were rewetted to 90% WFPS, apart from 30% WFPS which was wetted to 60%. A linear regression with a quadratic term has been fitted for the different treatments. This was fitted to the square root of the emissions and has been backtransformed.

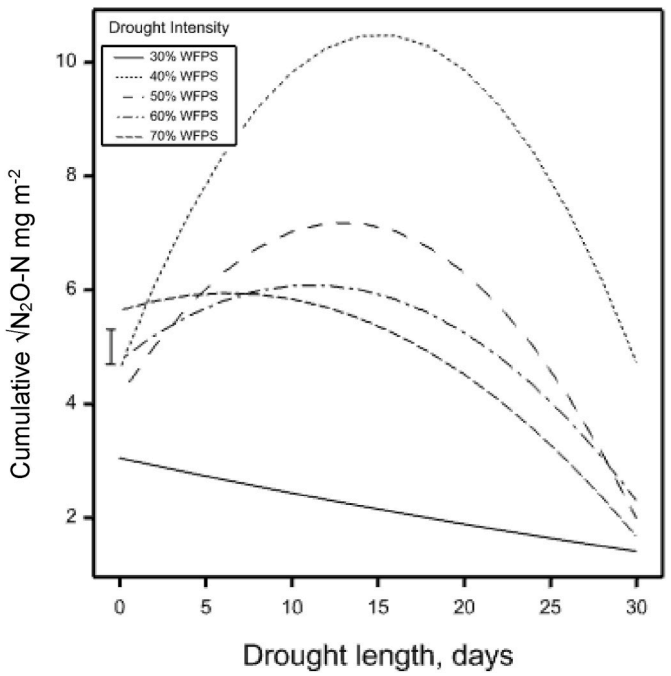

Fig. 3. Linear model with quadratic term of the relationship between the different treatments of drought intensity and drought length on the X axis, and the response in square root of cumulative N_2O–N emissions mg m^{-2} 14 days post wetting. All treatments were rewetted to 90% WFPS, apart from 30% WFPS which was wetted to 60%. The error bar represents the average standard error (1.44) of the predicted responses from the model.

square rooting the cumulative emissions for each treatment with an offset of +2, due to some of the samples and timepoints before wetting having a small negative value.

A two-way ANOVA was run with the N_2O emissions data as the Y variate, and days from wetting and the wetting treatments (pre-dry or pre-wet) as the treatment factors. However, there were two data sets for the emissions data because only a subset of samples were used for microbial and chemical analysis, but N_2O emissions were measured from all the cores. The gas data from all the soil cores is shown in the supplementary material. The same two way ANOVA was used to determine differences in the soil chemistry, and soil microbiology.

3. Results

3.1. Drought impact curve gas data

The final model allowed for different slopes and quadratic effects of drought length for each drought intensity. This model explained a significant proportion of the total variation in the data ($R^2 = 0.72$, $p \leq 0.001$). The length of drought had a significant quadratic relationship with the square root of the N_2O emissions post wetting ($p \leq 0.001$) as well as an overall downward linear trend ($p \leq 0.001$). Comparing across all the drought lengths the different wetting intensities had a significant impact on emissions ($p \leq 0.001$, 5 treatments, $n = 16$), and cumulative emissions were larger the greater the drought intensity (see Fig. 2). Although, out of the treatments that were wetted to 90%, only the 40–90% (mean 73.68 ± 9.17 mg m^{-2}) was significantly larger, with the other treatments not statistically different (LSD = 13.84 mg m^{-2}, 50–90% mean 35.84 ± 7.02 mg m^{-2} 60–90% mean 28.44 ± 28.44 mg m^{-2} and 70–90% mean 26.57 ± 5.52 mg m^{-2}). The 30–60% (mean 5.57 ± 1.26 mg m^{-2}) had the lowest emissions and this was significantly different to all other wetting treatments.

The response to drought length followed a quadratic curve (see Figs. 2 and 3), with the largest emissions observed between 10 and 15 days of drought. Overall, there was a downward linear trend, with the longest drought durations producing the lowest emissions, which was true for all drought intensities. There is insufficient evidence to suggest that the degree of the downward linear trend changes according to the drought intensity ($p = 0.19$), however the shape of the response to drought length was different according to the drought intensity ($p = 0.003$). This is noticeable in Figs. 2 and 3, with the higher initial WFPS treatments showing a shallower, less pronounced curvature. The daily N_2O flux data can be found in the supplementary material.

3.2. N_2O emissions for N cycling analysis

Analyzing N_2O–N fluxes at all the timepoints using all the replicates revealed that the pre-dry treatment ($n = 126$, untransformed mean N_2O flux 2.21 ± 0.51 mg m^{-2} day^{-1}) was statistically different than the pre-wet treatment ($n = 126$, untransformed mean N_2O flux 0.55 ± 0.12 mg m^{-2} day^{-1}) $p = 0.001$ (see Supplementary Fig. 1). For samples that were destructively sampled, the pre-dry treatment ($n = 26$, untransformed mean N_2O flux 3.18 ± 1.31 mg m^{-2} day^{-1}) was significantly different than the pre-wet treatment ($n = 26$, untransformed mean N_2O flux 1.06 ± 0.61 mg m^{-2} day^{-1}) $p = 0.037$. Peak emissions in the pre-dry treatment occurred on day 2 ($n = 3$, untransformed mean N_2O flux 7.32 ± 3.31 mg m^{-2} day^{-1}), and the peak emissions in the pre-wet treatment also occurred on day 2 ($n = 12$, untransformed mean N_2O flux 3.94 ± 2.03 mg m^{-2} day^{-1}). The untransformed cumulative emissions for the pre-dry soil post wetting were 19.04 mg m^{-2}, which was more than 3 times that of the pre-wet soil post wetting (6.47 mg m^{-2}).

The soils N_2O flux in relation to days from rewetting regardless of treatment, (time points in days, −4 n = 36, −3 n = 36, −2 n = 36, −1 n = 36, 0 n = 30, +1 n = 24, +2 n = 18, +3 n = 12, +4 n = 6, +5 n = 6, +6 n = 6,+7 n = 6) was significantly different $p = 0.015$. And the interaction between days from rewetting and treatment was not significantly different for the N_2O flux ($n = 6$ at each time point in days, −14. −1, 0, +1, +2, +3 and + 7) $p = 0.131$. See Fig. 4.

3.3. Soil chemistry

The soil's pH was significantly different between the pre-dry treatment ($n = 9$, mean pH 5.99 ± 0.05) and the pre-wet treatment ($n = 9$, mean pH 6.17 ± 0.05) $p = 0.012$. The soil's pH in relation to days from rewetting regardless of treatment, ($n = 6$ at each time point in days, 0, +1, +2, +3 and + 7) was significantly different $p = 0.030$. The interaction between days from rewetting and treatment was not significantly different for pH ($n = 3$ at each time point in days, 0, +1, +2, +3 and + 7) $p = 0.537$.

The soil's TON concentrations was not significantly different between the pre-dry treatment ($n = 26$, mean TON 0.020 ± 0.002 mg g^{-1}) and the pre-wet treatment ($n = 26$, mean TON 0.024 ± 0.002 mg g^{-1}) $p = 0.112$. The soil's TON in relation to days from rewetting regardless of treatment, ($n = 6$ at each time point in days, −14, −1, 0, +1, +2, +3 and + 7) was significantly different $p = 0.001$. The interaction between days from rewetting and treatment was not significantly different for TON ($n = 3$ at each time point in days, −14. −1, 0, +1, +2, +3 and + 7) $p = 0.856$. See Fig. 5.

The soil's NH_4^+ concentrations were significantly different between the pre-dry treatment ($n = 26$, mean NH_4^+ 0.036 ± 0.003 mg g^{-1}) and the pre-wet treatment ($n = 26$, mean NH_4^+ 0.031 ± 0.002 mg g^{-1}) $p < 0.001$. The soil's NH_4^+ in relation to days from rewetting regardless of treatment, ($n = 6$ at each time point in days, −14. −1, 0, +1, +2, +3 and + 7) was significantly different $p < 0.001$. The interaction between days from rewetting and treatment was significantly different for NH_4^+ ($n = 3$ at each time point in days, −14, −1, 0, +1, +2, +3 and + 7) $p = 0.009$. See Fig. 5.

The soil's DOC concentrations was significantly different between the pre-dry treatment ($n = 26$, mean DOC 0.297 ± 0.013 mg g^{-1}) and the pre-wet treatment ($n = 26$, mean DOC 0.256 ± 0.015 mg g^{-1}) p =

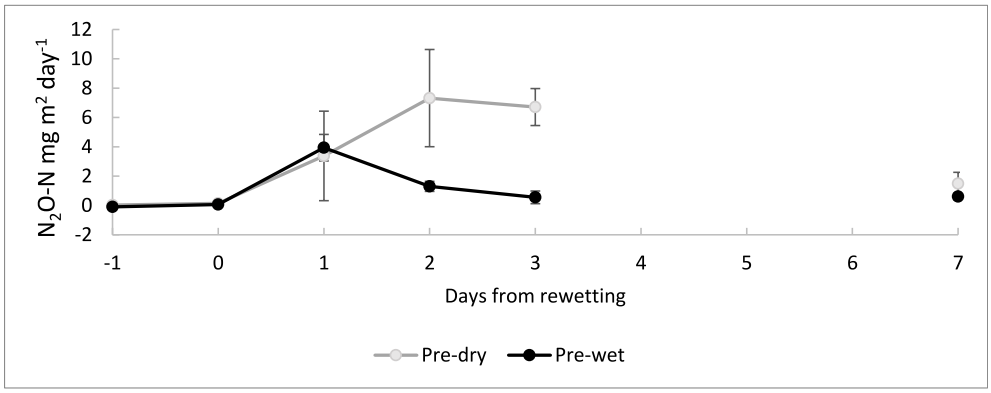

Fig. 4. N_2O fluxes for the destructively sampled soil cores throughout the experiment. The pre-dry soil was kept at 40% WFPS, and wetted to 90% WFPS at day 0, pre-wet soil was kept at 70% WFPS, and wetted to 90% WFPS at day 0. Error bars represent standard error, n = 3.

0.03. The soil's DOC in relation to days from rewetting regardless of treatment, (n = 6 at each time point in days, −14, −1, 0, +1, +2, +3 and + 7) was significantly different p = 0.026. The interaction between days from rewetting and treatment was not significantly different for DOC (n = 3 at each time point in days, time points −14, −1, 0, +1, +2, +3 and + 7) p = 0.939. See Fig. 5.

3.4. Microbiology

Due to the large quantity of variables measured, the ANOVA table for DNA and RNA as well a summary of the data can be found in the supplementary material. In summary, the soil's DNA concentrations in terms of copy number per gram of dry soil for targeted genes (Supplementary Table 1) was significantly different between the pre-dry treatment and the pre-wet treatment (n = 26, time points in days, −14, −1, 0, +1, +2, +3 and + 7) for *16S B* ($p < 0.026$), *ITS* ($p < 0.001$) and *16S P* ($p < 0.007$), see Fig. 6 and Supplementary Table 2. In terms of days from rewetting, (n = 6 per day, time points −14, −1, 0, +1, +2, +3 and + 7) the following genes were significantly different: *16S A* ($p < 0.001$), *16S P* ($p < 0.015$), *ITS* ($p < 0.001$), *AmoA* ($p < 0.027$), *nirS* ($p < 0.0390$), *nirK* ($p < 0.017$), *nosZI* ($p < 0.007$), *nosZII* ($p < 0.001$), *comaB* ($p < 0.026$). In terms of the interaction between treatment and days (n = 3), only *ITS* was significantly different ($p < 0.001$).

The following gene transcripts (*AmoA, AmoB, nirS, nirK, nosZI, nosZII* and *norB)* were below the level of detection under these experimental conditions despite increasing the total amount of total RNA per PCR from 20 ng to 40 ng. Only Pseudomonas 16S rRNA gene transcripts, in terms of copy number per gram of dry soil, was significantly different between the pre-dry treatment and the pre-wet treatment (n = 15, time points in days, 0, +1, +2, +3 and + 7), *16S P* ($p = 0.021$) see Fig. 6 and Supplementary Table 2. In terms of days since rewetting, (n = 6 per day, time points in days, 0, +1, +2, +3 and + 7) the following gene transcripts were significantly different: *16S B* ($p = 0.021$), *16S A* ($p = 0.006$), *16S P* ($p < 0.001$), and *ComaB* ($p = 0.015$). In terms of the interaction between treatment and days (n = 3), *16S A* ($p = 0.012$) and ComaB ($p < 0.001$) were significantly different.

4. Discussion

This study consisted of two experiments, the first induced a range of antecedent treatments to determine the relationship between the size of an N_2O hot moment, and the drought length and drought intensity. The second experiment consisted of two treatments selected from the first experiment to elucidate how changes in soil chemistry and microbiology are driving the higher N_2O emissions post wetting. All treatments had an increase in daily N_2O emissions post wetting, and all treatments besides the 30%–60% WFPS treatment had a response that is typical of a hot moment from an unfertilized soil (Priemé and Christensen, 2001; Molodovskaya et al., 2012; Harrison-Kirk et al., 2013; Leitner et al., 2017; Barrat et al., 2021), wherein the daily emissions post wetting are >10 times the daily emissions before rewetting, over a 24–72 h period.

4.1. Drought impact curves

Drought length had a significant impact on the size of a N_2O hot moment, which is shown in Figs. 2 and 3, which we have defined as drought impact curves. The relationship that was observed was an inverted U shape, which is different to what was hypothesized (H_1), as it was assumed to have an increasing positive linear response as drought intensity has been shown to have this relationship (Priemé and Christensen, 2001; Ruser et al., 2006; Harrison-Kirk et al., 2013). Because the size of hot moment increases and then decreases according to drought length, this has significant implications for predicting the size of hot moments, as future studies which will need to determine the degree of moderate drought that will induce the greatest response, and define the limit for when a severe drought will rapidly reduce the emissions post wetting. Moreover, while the model shows a continued curve (see Fig. 3), it is clear from the raw data (see Supplementary Table 3) that as predicted (H_2) it took >6 days of drying before the hot moment started to increase in size, therefore future incubation studies will also need to determine the minimum drought period required before a hot moment could be induced.

We assumed that increasing drought intensity would increase the size of the hot moment (H_3); with the caveat that the final WFPS after rewetting needed to create a sufficiently anaerobic soil for there to be a large increase in N_2O emissions (H_4), and this was observed, with very low emissions from the 30%–60% WFPS treatment comparative to the other treatments. It is possible that this explains the results of studies like Pezzolla et al. (2019) and Owens et al. (2016) that induced drying and wetting cycles, but did not observe an N_2O hot moment, as the treatments never created an ideal environment for anaerobic respiration. However, in our study the increase in cumulative emissions as the drought intensity was increased was only significant for 40–90% ($p < 0.05$), the other treatments that were wetted to 90% were not significantly different from each other. The large difference in emissions from the 40% WFPS treatment compared to the others, could be due to an important shift in the soil's matric potential, as although water content is linear, matric potential is extremely non-linear, and the 40% WFPS treatment could be situated either side of the soils capillary fringe (Whalley et al., 2013). Although the 50%, 60% and 70% drought curves were statistically similar, we predicate that this is an artifact of the statistical and experimental approach. We therefore hypothesize that if this experiment were repeated with replicates for the drought length treatments between 10 and 20 days, the fitted curves would be more accurate and there would be more power to detect differences between wetting intensities.

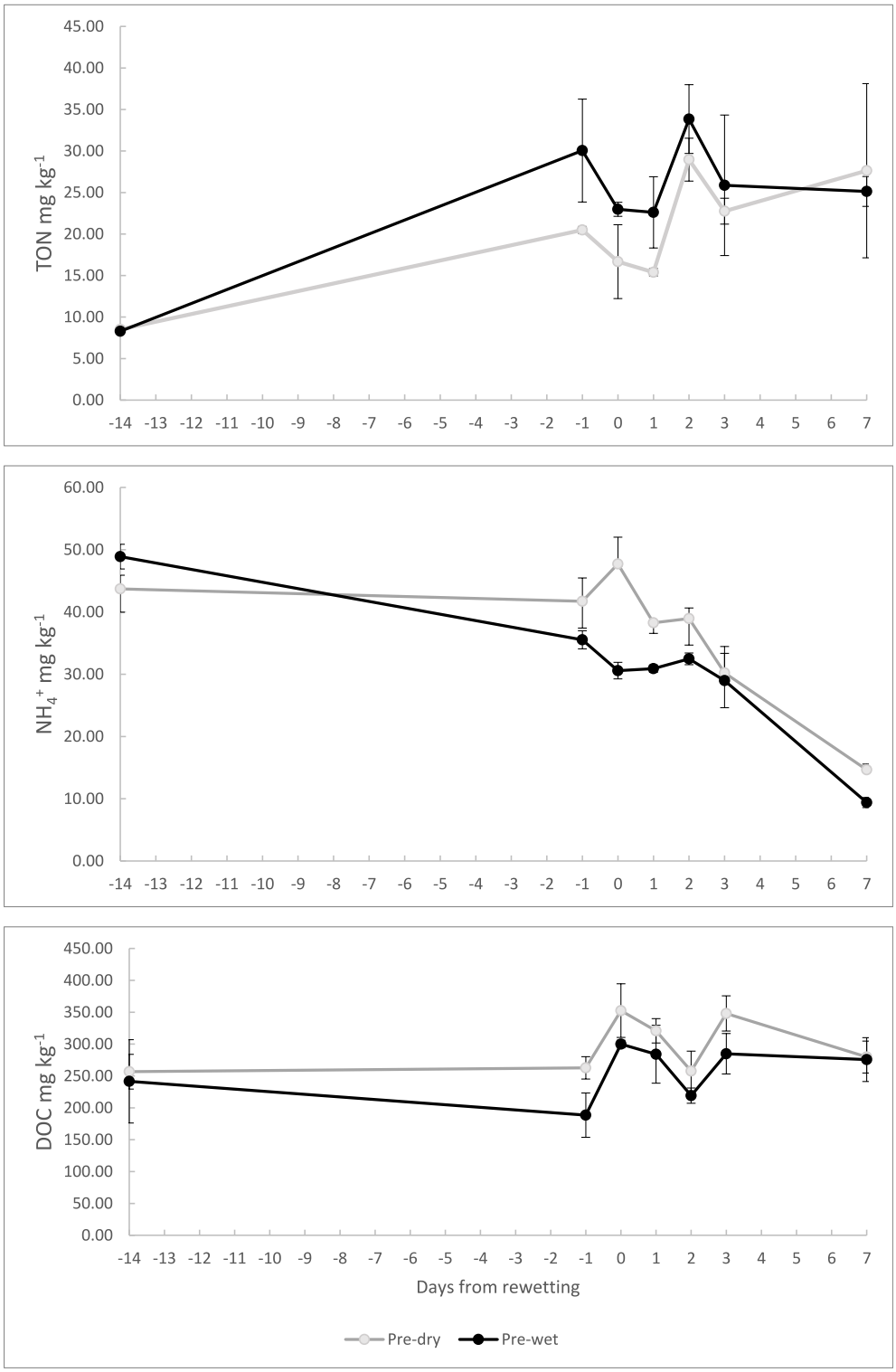

Fig. 5. Soil chemistry changes for total extractable oxidized nitrogen (TON), ammonium ($NH4^{+}$) and dissolved organic carbon (DOC) on a dry soil basis, from experiment 2. The pre-dry soil was kept at 40% WFPS, and wetted to 90% WFPS at day 0, pre-wet soil was kept at 70% WFPS, and wetted to 90% WFPS at day 0. Error bars represent standard error, n = 3 for each treatment at each timepoint.

4.2.1. Changes in microbiology

For the soil cores that were destructively sampled, we observed a decrease in the abundance of most of the functional genes from DNA post wetting, which could be due to cell death as the absolute change in water potential, and the rate of change over time is a stress event that may cause cell death and cell lysis (Schimel, 2018). Clark and Hirsch (2008), showed that the viability of culturable bacteria and extractable DNA decreased in soils that were air dried at ambient conditions prior to long term archiving. This supports the decrease in functional genes and viability of bacterial soil communities shown in this study under drought conditions. As expected (H_5) there was no difference in the quantity of functional N cycling genes between the pre-dry and pre-wet treatments, but there were two population markers that might account for the differences in emissions, fungal *ITS* and pseudomonas *16S* (see Fig. 6).

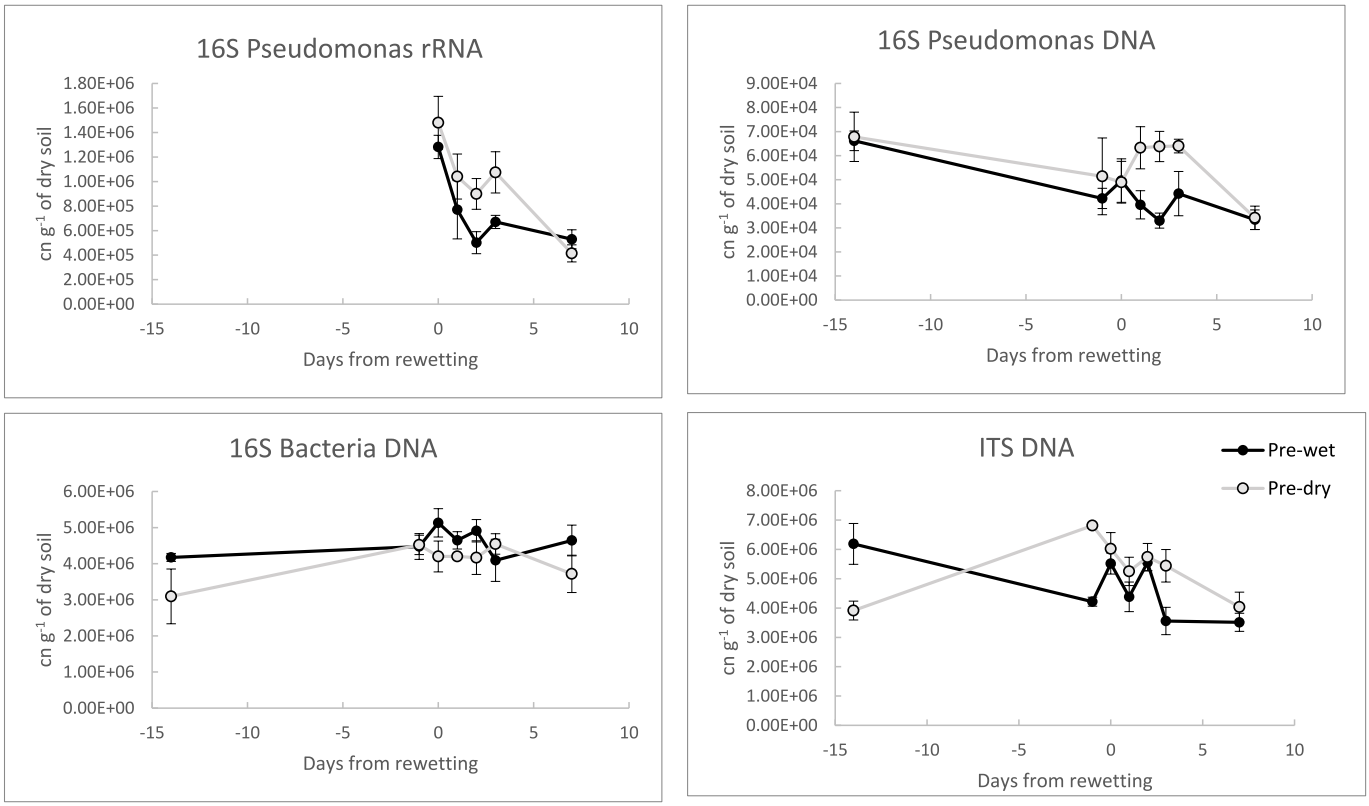

Fig. 6. Change in different functional genes throughout the experiment which were significantly different ($p < 0.05$) according to treatment. Soil was rewetted at day 0, error bars represent standard error, n = 3.

Fungal *ITS* decreased less in the pre-dry soil, and it is well established that fungi are better at surviving changing water potentials (Schimel, 2018; Barnard et al., 2013; Evans and Wallenstein, 2012). Pseudomonas *16S* increased post wetting in the pre-dry after a slight decrease. Increases in rRNA population markers could either indicate rapid growth or rapid activity, and many pseudomonas are well known relatively fast growing denitrifiers (Davies et al., 1989), so this population could be the source of N_2O from their growth and activity. Moreover, the delay in the response of this population marker, matches the delay in the peak emissions.

4.2.2. Hot moments and the key nitrogen pathways

Although we hypothesized (H_7) that the transcriptional data could explain the differences in emissions, the lack of differences between the two treatments in terms of mRNA and to some extent DNA, suggests that the active processes are more complex than initially thought. Firstly, N_2O does not seem to be produced due to an increase in anaerobic denitrification transcript or DNA abundance via NO_2^- (*nir*S, *nir*K) or NO (*nor*B). Secondly, it does not seem to be due to a change in the rate of reduction of N_2O to N_2 (*nos*ZI, *nos*ZII). Chemodenitrification is possible if there are high concentrations of NH_2OH or NO_2^- and low concentrations of O_2, however the differences in TON between the treatments were not statistically significant, and it is typically observed after N is artificially added (Liu et al., 2018, 2019; Anderson and Levine, 1986). Moreover, it took several days to reach the peak N_2O emissions, whereas an abiotic source would produce a peak within several hours (Leitner et al., 2017). Wang et al. (2017), however demonstrated that within short-term waterlogged soils, although the relative abundance of denitrifiers within the soil did not significantly change, the composition of these microbial denitrifiers did change and potentially to more active populations. Analysis of shifts in microbial community structure were not within the scope of this study. However, an increase in microbial activity, although not necessarily picked up in gene abundance, should have been seen in increases in associated N-cycling transcripts, which were not observed in this study.

There were slight and significant differences in NH_4^+ which contrary to our hypothesis (H_6) could be indicating the key process (see Fig. 5). In this study some form of nitrifier activity seems the most probable explanation for the differences in N_2O emissions, as NH_4^+ decreased rapidly in both treatments during rewetting (Leitner et al., 2017). There was no significant difference between treatments for DNA or RNA in terms of *AmoA*, *AmoB*, and COMAMMOX *AmoB*. Caranto and Lancaster (2017) propose that NH_4^+ is oxidized to NO via NH_2OH, which can produce N_2O as a non-enzymatic by-product. This could be likely given the lack of *nirK, nirS* or *norB* activity, and it is possible that NH_4^+ is being oxidized to NH_2OH using an, as yet, undetermined gene. It is also possible that the reduction in NH_4^+ is not related to the increase in N_2O, and instead it is being utilised via the ANNAMOX pathway, producing N_2. This seems unlikely given that the literature has shown the important contribution NH_4^+ addition can have on the emission pulse following rewetting (Slessarev et al., 2021; Leitner et al., 2017; Heil et al., 2016; Zhu et al., 2013).

If NH_4^+ is being oxidized to NH_2OH, then further oxidation using hydroxylamine oxidoreductase (*HAO*) can produce large amounts of N_2O either via NO or straight to N_2O, and this can occur under anaerobic conditions (Caranto and Lancaster, 2017; Caranto et al., 2016; Otte et al., 1999; Hooper and Terry, 1979). Moreover, there is increasing evidence that nitrifiers under oxic shock will utilise NO_2^- and NH_2OH (Liu et al., 2018, 2019; Wrage-Mönnig et al., 2018). Caranto et al. (2016) propose a pathway that involves the oxidation of NH_2OH which produces N_2O, NO and NO_2^- as a by-product. This could be the case in this study, however we did not measure *HAO* transcription levels or its genetic abundance. Besides NH_2OH oxidation, there is also the possibility of nitrifier denitrification which typically involves the use of NO_2^- as the electron acceptor. It is predicted that NO_2^- builds up in dry soil and therefore could be readily utilised upon rewetting (Liu et al., 2018).

However, we measured no changes in *nirS* or *nirK* in terms of gene expression or in terms of functional gene abundance. In this study the pH was significantly different between treatments, however the difference is biological negligible in terms of affecting N_2O (less than 0.2) (Šimek and Cooper, 2002). The difference was likely caused by the continuous rewetting of the pre-wet samples.

4.2. Future studies and the use of metabolomics

Given the inconclusive evidence from this study, and the current pool of literature, we suggest a new approach for future studies, which is based on the methods for exploring a similar phenomena known as the Birch effect, where rewetted soils produce a pulse in CO_2 emissions.

The work of Warren (Warren, 2020, Warren, 2014a; Warren, 2014b) has used metabolomics to investigate the Birch effect, and has made significant progress in understanding how osmolytes fuel the emissions pulse. This could be a relevant analogue for N_2O hot moments, as the use of nitrogenous osmolytes is common in cell cultures and in soil (Schimel, 2018; Warren, 2014). Studies exploring the Birch effect and osmolyte accumulation have shown that it does not follow a linear response to drying, as accumulation of osmolytes is observed under moderate dryness, but extreme drying conditions seems to supress this strategy (Warren, 2016; Kakumanu et al., 2013). This is because osmolyte accumulation is costly and at certain water potentials as it no longer provides effective osmoregulation. This matches the inverted U shape response in emissions observed in study. It is therefore possible in this study that the time delay could indicate the mineralisation and then catabolism of osmolytes, but this will need to be further investigated This study used only one soil type, and this soil was sieved and had no vegetation cover, therefore future studies using different soil types with plant cover might observe a different a response to changing antecedent moisture conditions.

5. Conclusion

In summary, this study outlined the relationship between the size of the N_2O response post wetting, and the antecedent conditions of drought length and intensity. For this grassland soil, there is an inverted U shaped response in terms of drought days, with 10–15 days of drying showing the largest response, while 0 and 30 days show the smallest. We suggest a 2-stage dormancy strategy to explain this, where microbes under dry conditions store osmolytes which are catabolised upon rewetting, however at prolonged negative water potentials this strategy is no longer effective, and so they enter a deeper state of dormancy, resulting in a dormant microbial community that can no longer rapidly respond to the changing water potential. From this experiment, we hypothesize that the source of the N_2O emissions is from the mineralisation of osmolytes. Moreover, given the delayed response after rewetting, and the inverted U shaped curve in terms of drought length, it seems likely that the majority of emissions are of biological origin. Furthermore, given the lack of transcriptional activity in our soil in terms of *nirK*, *nirS*, *amo* and *norB*, we suggest that pathways proposed by Caranto et al. (2016) and Caranto and Lancaster (2017) seem probable where N_2O is a product of nitrifier activity from the oxidation of NH_2OH.

Given the results of this study are from a single sieved soil type with no vegetation cover, further work repeating this study's methodology will be necessary to fully elucidate the relationship between drought length, wetting intensity, and the size of the N_2O hot moment. If the hot moment is driven by NH_4^+ then plants using this pool could reduce the size of the hot moment, especially if the soil has a more connected macro pore structure from roots and a different soil texture with less clay (Ruser et al., 2006; Harrison-Kirk et al., 2013).

Funding

Rothamsted Research is core funded by the UK Biotechnology and Biological Sciences Research Council (BBSRC) (project codes BBS/E/C/000I0310 and BS/E/C/000I0320). This research is part of a PhD supported by the STARs studentship (NE/R010218/1).

Data sharing and data accessibility statement

Data from this experiment is shared in the supplementary material.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Alice Simonato for creating a sample holder for the soils cores, and for adapting the soil core lids for gas sampling, and Louise Olde for helping with some of the sampling days. The authors would also like to thank the soils training and research (STARs) studentship, Rothamsted Research, and the School of Natural Sciences at Bangor University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108606.

References

Anderson, I.C., Levine, J.S., 1986. Relative rates of nitric oxide and nitrous oxide production by nitrifiers, denitrifiers, and nitrate respirers. Applied and Environmental Microbiology 51, 938–945.
Avery, B.W., 1973. Soil classification in the soil survey of England and wales. Journal of Soil Science 24, 324–338.
Baggs, E.M., 2011. Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction. Current Opinion in Environmental Sustainability 3, 321–327.
Barnard, R.L., Blazewicz, S.J., Firestone, M.K., 2020. Rewetting of soil: revisiting the origin of soil Co2 emissions. Soil Biology and Biochemistry 147, 107819.
Barnard, R.L., Osborne, C.A., Firestone, M.K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. The ISME Journal 7, 2229–2241.
Barrat, H.A., Charteris, A.F., Le Cocq, K., Abadie, M., Clark, I.M., Chadwick, D.R., Cardenas, L., 2021. N_2O hot moments were not driven by changes in nitrogen and carbon substrates or changes in N cycling functional genes. European Journal of Soil Science 73 (1), E13190.
Barrat, H.A., Evans, J., Chadwick, D.R., Clark, I.M., Le Cocq, K., Cardenas, L.M., 2020. The impact of drought and rewetting on N_2O emissions from soil in temperate and Mediterranean climates. European Journal of Soil Science 1–13.
Bergstermann, A., Cárdenas, L., Bol, R., Gilliam, L., Goulding, K., Meijide, A., Scholefield, D., Vallejo, A., Well, R., 2011. Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N_2O during denitrification. Soil Biology and Biochemistry 43, 240–250.
Burke, E.J., Perry, R.H.J., Brown, S.J., 2010. An extreme value analysis of UK drought and projections of change in the future. Journal of Hydrology 388, 131–143.
Caranto, J.D., Lancaster, K.M., 2017. Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. Proceedings of the National Academy of Sciences of the United States of America 114, 8217–8222.
Caranto, J.D., Vilbert, A.C., Lancaster, K.M., 2016. Nitrosomonas europaea cytochrome P460 is a direct link between nitrification and nitrous oxide emission. Proceedings of the National Academy of Sciences 113, 14704–14709.
Davies, K.J.P., Lloyd, D., Boddy, L., 1989. The effect of oxygen on denitrification in paracoccus denitrificans and Pseudomonas aeruginosa. Microbiology 135, 2445–2451.
De Sosa, L.L., Glanville, H.C., Marshall, M.R., Williams, A.P., Abadie, M., Clark, I.M., Blaud, A., Jones, D.L., 2018. Spatial zoning of microbial functions and plant-soil nitrogen dynamics across a riparian area in an extensively grazed livestock system. Soil Biology and Biochemistry 120, 153–164.
Dodd, R.J., Chadwick, D.R., Harris, I.M., Hines, A., Hollis, D., Economou, T., Gwynn-Jones, D., Scullion, J., Robinson, D.A., Jones, D.L., 2021. Spatial co-localisation of extreme weather events: a clear and present danger. Ecology Letters 24, 60–72.
Evans, S.E., Wallenstein, M.D., 2012. Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry 109, 101–116.

Harrison-Kirk, T., Beare, M.H., Meenken, E.D., Condron, L.M., 2013. Soil organic matter and texture affect responses to dry/wet cycles: effects on carbon dioxide and nitrous oxide emissions. Soil Biology and Biochemistry 57, 43–55.
Heil, J., Vereecken, H., Brüggemann, N., 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. European Journal of Soil Science 67, 23–39.
Hooper, A.B., Terry, K.R., 1979. Hydroxylamine oxidoreductase of Nitrosomonas: production of nitric oxide from hydroxylamine. Biochimica et Biophysica Acta (BBA) - Enzymology 571, 12–20.
Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology and Biochemistry 38, 991–999.
Kakumanu, M.L., Cantrell, C.L., Williams, M.A., 2013. Microbial community response to varying magnitudes of desiccation in soil: a test of the osmolyte accumulation hypothesis. Soil Biology and Biochemistry 57, 644–653.
Leitner, S., Homyak, P.M., Blankinship, J.C., Eberwein, J., Jenerette, G.D., Zechmeister-Boltenstern, S., Schimel, J.P., 2017. Linking NO and N_2O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils. Soil Biology and Biochemistry 115, 461–466.
Liu, S., Schloter, M., Brüggemann, N., 2018. Accumulation of NO_2 during periods of drying stimulates soil N_2O emissions during subsequent rewetting. European Journal of Soil Science 69, 936–946.
Liu, S., Schloter, M., Hu, R., Vereecken, H., Brüggemann, N., 2019. Hydroxylamine contributes more to abiotic N_2O production in soils than nitrite. Frontiers in Environmental Science 7.
Mcclain, M.E., Boyer, E.W., Dent, C.L., Gergel, S.E., Grimm, N.B., Groffman, P.M., Hart, S.C., Harvey, J.W., Johnston, C.A., Mayorga, E., Mcdowell, W.H., Pinay, G., 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6, 301–312.
Molodovskaya, M., Singurindy, O., Richards, B.K., Warland, J., Johnson, M.S., Steenhuis, T.S., 2012. Temporal variability of nitrous oxide from fertilized croplands: hot moment analysis. Soil Science Society of America Journal 76, 1728–1740.
Otte, S., Schalk, J., Kuenen, J.G., Jetten, M.S.M., 1999. Hydroxylamine oxidation and subsequent nitrous oxide production by the heterotrophic ammonia oxidizer Alcaligenes faecalis. Applied Microbiology and Biotechnology 51, 255–261.
Owens, J., Clough, T.J., Laubach, J., Hunt, J.E., Venterea, R.T., Phillips, R.L., 2016. Nitrous oxide fluxes, soil oxygen, and denitrification potential of urine- and non-urine-treated soil under different irrigation frequencies. Journal of Environmental Quality 45, 1169–1177.
Pendergrass, A.G., Knutti, R., 2018. The uneven nature of daily precipitation and its change. Geophysical Research Letters 45 (11), 980-11,988.
Pezzolla, D., Cardenas, L.M., Mian, I.A., Carswell, A., Donovan, N., Dhanoa, M.S., Blackwell, M.S.A., 2019. Responses of carbon, nitrogen and phosphorus to two consecutive drying–rewetting cycles in soils. Journal of Plant Nutrition and Soil Science 182, 217–228.
Priemé, A., Christensen, S., 2001. Natural perturbations, drying–wetting and freezing–thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from farmed organic soils. Soil Biology and Biochemistry 33, 2083–2091.
Ruser, R., Flessa, H., Russow, R., Schmidt, G., Buegger, F., Munch, J.C., 2006. Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. Soil Biology and Biochemistry 38, 263–274.
Schimel, J.P., 2018. Life in dry soils: effects of drought on soil microbial communities and processes. Annual Review of Ecology, Evolution and Systematics 49, 409–432.
Šimek, M., Cooper, J.E., 2002. The influence of soil ph on denitrification: progress towards the understanding of this interaction over the last 50 years. European Journal of Soil Science 53, 345–354.
Slessarev, E.W., Greene, A.C., Homyak, P.M., Ying, S.C., Schimel, J.P., 2021. High resolution measurements reveal abiotic and biotic mechanisms of elevated nitric oxide emission after wetting dry soil. Soil Biology and Biochemistry 160, 108316.
Venterea, R.T., Petersen, S.O., De Klein, C.A.M., Pedersen, A.R., Noble, A.D.L., Rees, R. M., Gamble, J.D., Parkin, T.B., 2020. Global Research Alliance N2o chamber methodology guidelines: flux calculations. Journal of Environmental Quality 49, 1141–1155.
Vsn, I., 2020. Genstart for Windows 20th Edition. Vsn International, Hemel Hempstead, Uk.
Warren, C.R., 2014. Response of organic N monomers in a sub-alpine soil to a dry–wet cycle. Soil Biology and Biochemistry 77, 233–242.
Warren, C.R., 2016. Do microbial osmolytes or extracellular depolymerisation products accumulate as soil dries? Soil Biology and Biochemistry 98, 54–63.
Whalley, W.R., Ober, E.S., Jenkins, M., 2013. Measurement of the matric potential of soil water in the rhizosphere. Journal of Experimental Botany 64, 3951–3963.
Wrage-Mönnig, N., Horn, M.A., Well, R., Müller, C., Velthof, G., Oenema, O., 2018. The role of nitrifier denitrification in the production of nitrous oxide revisited. Soil Biology and Biochemistry 123, A3–A16.
Zhu, X., Burger, M., Doane, T.A., Horwath, W.R., 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N2o and No under low oxygen availability. Proceedings of the National Academy of Sciences of the United States of America 110, 6328–6333.