Rothamsted Repository Download

A - Papers appearing in refereed journals

The publisher's version can be accessed at:

- https://doi.org/10.1016/j.soilbio.2022.108606

The output can be accessed at: https://repository.rothamsted.ac.uk/item/98861/the-impact-of-drought-length-and-intensity-on-n-cycling-gene-abundance-transcription-and-the-size-of-an-n2o-hot-moment-from-a-temperate-grassland-soil.

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

H.A. Barrat[1], I.M. Clark[2], J. Evans[3], D.R. Chadwick[4], L. Cardenas[1]

[1]Rothamsted Research, Sustainable Agriculture Sciences Department, Devon, North Wyke, UK

[2]Rothamsted Research, Sustainable Agriculture Sciences Department, Hertfordshire, Harpenden, UK

[3]Rothamsted Research, Computational and Analytical Sciences, Hertfordshire, Harpenden, UK

[4]School of Natural Sciences, Bangor University, Bangor LL57 2UW, U.K.

Corresponding author: Harry Barrat, harry.barrat@rothamsted.ac.uk, Rothamsted Research, Sustainable Agriculture Sciences Department, Devon, North Wyke, UK

Highlights:

1. A greater drying intensity results in larger N_2O emissions if soil is sufficiently rewetted
2. Drought length and the size of N_2O hot moments are negatively related and non-linear
3. We observed no significant differences in transcription activity for N cycling genes
4. We suggest a two step drought response to explain differences in N_2O emissions

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% to 90%, 60% to 90%, 70% to 90%, and 30% to 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% to 60% WFPS treatment. In terms of drought intensity, the 40% to 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.

1.0 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 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

86 relationship between drought length and the size of the hot moment (i.e. the magnitude of the

87 flux), and how this changes with the drought intensity in terms of the starting WFPS.

88 In addition, there is still uncertainty regarding the main nitrogen (N) cycling processes that are

89 dominant post wetting. While it seems probable that anaerobic denitrification is the main

90 process, considering the low oxygen content (Baggs, 2011), the classical assumption of

91 reduction from nitrate (NO_3^-) has been challenged by new research into nitrifier denitrification

92 (Wrage-Mönnig et al., 2018), chemodenitrification (Liu et al., 2018), and the possibility of

93 antecedent conditions affecting N_2O reductase (Bergstermann et al., 2011). Unfortunately the

94 lack of studies that control for antecedent conditions and then measure changes in relative

95 abundance of N-cycling transcripts and gene abundance *in-situ*, have inhibited a better

96 mechanistic understanding of the processes involved (Barrat et al., 2020). Barrat et al. (2021)

97 did attempt to discern differences according to functional gene abundance, but no explanatory

98 changes were discovered. There is growing evidence that differences in cell lysis and

99 osmolyte expulsion at the time of rewetting between treatments is unlikely to be the reason for

100 differences in emissions (Barnard et al., 2020, Schimel, 2018, Kakumanu et al., 2013).

101 Instead, the priming effect of the antecedent conditions on the microbial community could be

102 favoring a process that produces more N_2O than a wet control.

103 This study, using one soil type, is comprised of two experiments. The first experiment aimed

104 to reveal which drought durations and intensities produce the smallest and largest hot moment

105 responses. Following the conclusions of Barrat et al. (2020) we hypothesize that: (H_1), the

106 longer the drought, the larger the hot moment, however this effect will plateau; that (H_2), it will

107 take a minimum number of drying days before the largest hot moment will be observed; and

108 that (H_3), the more intense the drought the greater the hot moment. Also that (H_4), the WFPS

109 after rewetting will have a significant effect on emissions.

110 The second experiment aimed to reveal which key N cycling processes are dominant at the

111 time of rewetting. As previously work failed to discern differences according to gene

112 abundance and soil chemistry (Barrat et al., 2021), this study aimed to replicate that result

4

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.

2.0 Methods

2.1.1 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

139   and variation across a range of drought lengths and intensities, therefore replication at each

140   drought length (e.g. 3 replicates at 2 days) was not required as background variation could be

141   captured across the range of drought lengths and treatments (see section 2.6).

142   **2.1.2 Experimental design for the analysis of N processes**

143   Using the same grassland soil two antecedent moisture treatments were selected that were

144   shown to produce a hot moment from section 2.1.1. One set of 18 soil cores were kept at 40%

145   WFPS for 14 days, which we defined as the pre-dry treatment, and another set of 18 soil cores

146   were kept at 70% WFPS for 14 days, which we defined as the pre-wet treatment, both were

147   then rewetted to 90% WFPS and held there for 7 days. Destructive sampling of the cores for

148   chemical and biological analysis was informed by the daily N_2O emission data (see section

149   2.5 and 2.6), and at each time point 3 replicates from each treatment were sampled. Soil was

150   collected and prepared and emissions were measured in the same manner as section 2.1.1.

151   A visual summary of both experiments is outlined in figure 1.

152   **2.2.1 Soil collection and preparation**

153   Soil was collected from Rothamsted Research's Rowden grassland site at North Wyke,

154   England, at 9 randomly determined points from the 0 to 10 cm depth using a trowel. The soil

155   is a clayey pelostagnogley also known as a Stagni-vertic Cambisol, and it is mottled

156   throughout with greyish colours, see table 1 for other characteristics (Avery, 1973). Roots,

157   stones and vegetation were removed from the soil by hand, and it was air dried for 6 days,

158   reaching a moisture level of 28% WFPS, assuming a packing density of 0.8 g cm^{-3} and a

159   particle density of 2.43 g cm^{-3}. Then a mixed pool of soil was created and sieved through a 9

160   mm sieve. It was stored for 2 days at 4 °C before 9.44 kg of the air dried soil was weighed into

161   80 polyethylene bags (118 g per bag) representing each core. When the soil was initially put

162   into each bag it was wetted to 30% WFPS. Then each bag was wetted to half its starting

163   WFPS a day before packing the soil cores, and then a day later they were completely wetted

164   to the appropriate initial WFPS when the cores were packed. This was done to stagger the

165   rewetting and prevent an initial hot moment for soil cores starting at a higher WFPS. The 5

166   treatments with 16 different drought lengths resulted in 80 cores that were packed to a height

167   of 7.6 cm (Greiner Bio-One multipurpose container, 150 ml, metal screw cap, clear, aseptic,

168   item 225170), at a bulk density of 0.8 g cm^{-3}. The cores were packed by filling and compacting

169   them in thirds, so that the bulk density was consistent throughout the core. The bulk density

170   was selected as a replication of a previous experiment (Barrat et al., 2021).

171   For the second experiment, soil was collected again 10 days later from the same site, for the

172   packing of cores that were going to be destructively sampled. This pool of soil was air dried

173   for 3 days to reach a moisture level of 40% WFPS, which was determined by taking 3 x 50 g

174   samples each day to determine gravimetric moisture content and assuming a packing density

175   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

176   and stored for 2 days at 4 °C. Then 4.52 kg of the air dried soil was weighed and divided into

177   36 polyethylene bags (125.5 g per bag) representing each core. Each bag of soil that

178   represented the 70% WFPS treatment was wetted to 55% WFPS a day before packing the

179   soil cores, and then a day later they were completely wetted to the appropriate initial WFPS

180   when the cores were packed, to stagger the rewetting and prevent an initial hot moment. It is

181   worth noting that the second experiment was not a replication of the first, so drying times and

182   sampling times were different due to the different objectives and treatments.

183   **2.3 Incubation and N$_2$O measurements**

184   The soil cores were kept in a temperature-controlled room at approximately 18 °C to represent

185   a summer day, and throughout the experiment the cores were weighed daily and the WFPS

186   of each core was adjusted when needed to keep it constant, by adding deionized water

187   assuming weight loss due to evaporation. When measuring emissions, the cores were sealed

188   using modified metal screw caps containing two rubber septa, 3 cm apart, allowing the

189   headspace (49.3 cm^3 at 7.6 cm packing height) to be sampled via PTFE 3.2 mm tubing.

190   A Picarro cavity ringdown spectrometer G2508 was used to measure the N_2O concentrations.

191   The headspace for each core was sealed for 90 seconds before sampling, and then the flux

192   was measured for 90 seconds. The first 10 seconds of measurement were ignored to allow

193   circulation of the cores' headspace, and the following 80 seconds of measurement were used

194   to calculate a linear regression from T10 to T90 to generate a flux as outlined by Venterea et

195   al. (2020). This was converted from parts per million (ppm) into a concentration based on the

196   ideal gas law, the chambers dimensions, and the time of measurement (see equation 1).

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

198   ***Equation 1.*** *Used to calculate emissions in terms of grams per meter squared per day. Fppm*

199   *is the ppm flux, atm is the ambient air pressure, K is the air temperature in kelvin, R is the*

200   *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*

201   *chamber volume in m^3 and Cha is the chamber area in m^2, 0.76188 converts the flux from 1*

202   *minute 20 seconds to 1 minute, multiplying by 1440 converts it to a daily flux.*

203   Measurements were taken twice a day (approximately 2 hours apart), and an average from

204   the two samplings was taken for each core creating a daily flux. This was converted into the

205   cumulative emissions over 14 days for each treatment, by adding up all the daily emissions

206   for 14 days post wetting (see supplementary material for a summary of the data).

207   **2.4 Soil chemical analyses**

208   Soil chemical characteristics were determined by destructive sampling the soil cores on days:

209   -14, -1, 0, 1, 2, 3 and 7. Day 0 marks the day of rewetting, measurements were taken after the

210   soil was rewetted on this day. Dissolved organic carbon (DOC), total extractable oxidized

211   nitrogen (TON) and ammonium (NH_4^+), was determined on supernatant following 0.5 M

212   potassium sulphate (K_2SO_4) extractions with 10 g of fresh soil in 50 ml of extractant. Samples

213   were shaken for 30 minutes and then centrifuged (14,000 rpm, 30 minutes), the supernatant

214   was removed and frozen until later analysis (Jones and Willett, 2006). Inorganic N

215   concentrations were then determined using an Aquakem 250 discrete photometric analyzer.

216   DOC concentrations were determined using a Shimadzu TOC-L CPN total organic carbon

217   analyzer, the samples were diluted by a 10x dilution factor, to prevent precipitation in the

218   instrument. Gravimetric moisture was determined using 50 g samples in an oven at 105 °C for

219   24 hours. Soil pH was determined using a pH meter with a ratio of 5 g to 12 ml of deionized

220   water using a Jenway 3320 pH meter.

221   **2.5 DNA and RNA extractions and determination**

222   DNA was extracted from the same time points as the destructively sampled chemical analysis

223   (days -14, -1, 0, 1, 2, 3 and 7) whilst RNA was extracted from all time points post wetting (days

224   0, 1, 2, 3 and 7). Each time point had 3 true replicates per treatment, and each true replicate

225   consisted of 2 technical replicates. To test changes in functional gene abundance, 2 sub

226   samples of soil were taken per soil core replicate and frozen in 15 ml falcon tubes (~20 g of

227   soil per tube) using dry ice, and stored at -80 °C. The soil samples were then freeze-dried to

228   further preserve the integrity of the nucleic acid and improve homogenisation of the samples.

229   In summary, DNA was extracted from 84 soil samples, and RNA was extracted from 60 soil

230   samples.

231   DNA was extracted using and following the instructions in the DNeasy PowerSoil kit (Qiagen)

232   in batches of 24 (and one batch of 12), using 250 mg of freeze-dried soil. 50 µl of autoclaved

233   deionised water was added to each sample at the beginning of each extraction to rehydrate

234   the sample. The concentrations of DNA were measured using a Qubit dsDNA BR Assay Kit

235   (ThermoFisher) and the quality was assessed on a Nanodrop spectrophotometer

236   (ThermoFischer). Samples were then diluted to 20 ng µl^{-1} and stored at −80°C prior to qPCR

237   analysis.

238   RNA was extracted using the RNeasy PowerSoil Total RNA kit (Qiagen), in batches of 12,

239   using 2.0 g of freeze-dried soil. Samples were pre-weighed before the day of extraction using

240   a weighing spatula that was heated until it was red hot and then cooled in ethanol. We added

241 400 µl of autoclaved deionised water to each sample after adding phenol, to rehydrate the

242 sample and improve the extraction success. For acidic soils, we found that modifying step 7

243 by adding 0.75 ml of SR5 and 0.75 ml of SR3 (instead of 1.5 ml of SR3), dramatically increased

244 the extraction success, and so all extractions included this modified step. The RNA samples

245 were purified using the DNase Max kit (Qiagen), and the concentrations of RNA were

246 measured using a Qubit RNA BR Assay Kit (ThermoFisher) and the quality was assessed on

247 a Nanodrop spectrophotometer (ThermoFischer). Samples were then diluted to 20 ng µl^{-1} and

248 stored at −80°C prior to qPCR analysis.

249 For DNA samples, qPCR analyses were performed as described in de Sosa et al. (2018).

250 Briefly, using a 384 plate, each run contained 3 different genes, 24 blanks, 2 no-template

251 blanks per gene, 8 x 3 standards per gene, and 2 positive controls using a standard created

252 from a mix of grassland and arable soil. Specific primers (see supplementary table 1) were

253 employed to quantify gene abundance of microbial kingdoms and genes associated with N

254 cycling using the QuantiFast SYBR® Green PCR Kit (Qiagen) and a Biorad CFX384 Touch

255 Real-Time PCR Detection System. DNA copy numbers are represented on a per gram of dry

256 soil basis (cn g^{-1}). Results were standardised assuming 40 ng ul per well.

257 RT-qPCR analysis on RNA samples were performed using a 384 plate, each run contained 4

258 different genes, 2 blanks, 2 no-template blanks, 8 x 3 standards per gene, and 2 positive

259 controls using a standard created from a mix of grassland and arable soil. Specific primers

260 (see supplementary table 1) were employed to quantify gene abundance of microbial

261 kingdoms and genes associated with N cycling using the RT-QuantiFast SYBR® Green PCR

262 Kit (Qiagen) and a Biorad CFX384 Touch Real-Time PCR Detection System. The list of genes

263 analysed and the qPCR extraction efficiency for each gene is stated in supplementary table

264 1.

265 **2.6 Data treatment and statistics for the drought curves**

266   Genstat 20th edition was used for statistical analysis (VSN, 2020). The experimental data were

267   transformed to satisfy the normality and homogeneity of variance assumptions of the analysis

268   by square rooting the cumulative emissions for each treatment. Curves were then fitted to the

269   data to estimate the relationship between the drought days (on the X axis), and the cumulative

270   N_2O emissions post wetting (on the Y axis) for each drought intensity. These curves were

271   fitted using linear regression with a quadratic term included to capture the curvature of the

272   relationship (see figures 2 and 3). Sequential F tests were used to determine significance

273   ($p \leq 0.05$) of the model terms and therefore how complex the final model needed to be to

274   sufficiently describe the relationships.

275   A one-way ANOVA was used with the cumulative N_2O emissions data as the Y variate and

276   WFPS as the Treatment term and the number of days of drying as the block term. In order to

277   determine if on average the different WFPS intensities affected N_2O emissions (adjusting for

278   drought lengths). Cumulative emissions for each treatment were calculated by averaging the

279   emissions for each treatment for each day and totaling all the days post wetting.

280   **2.7 Data treatment and statistics for the analysis of N processes**

281   The N_2O experimental data were transformed to satisfy the normality and homogeneity of

282   variance assumptions of the analysis by square rooting the cumulative emissions for each

283   treatment with an offset of +2, due to some of the samples and timepoints before wetting

284   having a small negative value.

285   A two-way ANOVA was run with the N_2O emissions data as the Y variate, and days from

286   wetting and the wetting treatments (pre-dry or pre-wet) as the treatment factors. However,

287   there were two data sets for the emissions data because only a subset of samples were used

288   for microbial and chemical analysis, but N_2O emissions were measured from all the cores.

289   The gas data from all the soil cores is shown in the supplementary material. The same two

290   way ANOVA was used to determine differences in the soil chemistry, and soil microbiology.

291

3.0 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≤0.001). The length of drought had a significant quadratic relationship with the square root of the N_2O emissions post wetting (p≤0.001) as well as an overall downward linear trend (p≤0.001). Comparing across all the drought lengths the different wetting intensities had a significant impact on emissions (p≤0.001, 5 treatments, n=16), and cumulative emissions were larger the greater the drought intensity (see figure 2). Although, out of the treatments that were wetted to 90%, only the 40 to 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 to 90% mean 35.84 ± 7.02 mg m^{-2} 60 to 90% mean 28.44 ± 28.44 mg m^{-2} and 70 to 90% mean 26.57 ± 5.52 mg m^{-2}). The 30 to 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 figures 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 figures 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

318   different than the pre-wet treatment (n=126, untransformed mean N_2O flux 0.55 ± 0.12 mg m^{-2}

319   day^{-1}) p=0.001 (see supplementary figure 1). For samples that were destructively sampled,

320   the pre-dry treatment (n=26, untransformed mean N_2O flux 3.18 ± 1.31 mg m^{-2} day^{-1}) was

321   significantly different than the pre-wet treatment (n=26, untransformed mean N_2O flux 1.06 ±

322   0.61 mg m^{-2} day^{-1}) p=0.037. Peak emissions in the pre-dry treatment occurred on day 2 (n=3,

323   untransformed mean N_2O flux 7.32 ± 3.31 mg m^{-2} day^{-1}), and the peak emissions in the pre-

324   wet treatment also occurred on day 2 (n=12, untransformed mean N_2O flux 3.94 ± 2.03 mg

325   m^{-2} day^{-1}). The untransformed cumulative emissions for the pre-dry soil post wetting were

326   19.04 mg m^{-2}, which was more than 3 times that of the pre-wet soil post wetting (6.47 mg m^{-}

327   ^{2}).

328   The soils N_2O flux in relation to days from rewetting regardless of treatment, (time points in

329   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

330   n=6, +6 n=6,+7 n=6) was significantly different p=0.015. And the interaction between days

331   from rewetting and treatment was not significantly different for the N_2O flux (n=6 at each time

332   point in days, -14. -1, 0, +1, +2, +3 and +7) p=0.131. See figure 4.

333   **3.3 Soil chemistry**

334   The soil's pH was significantly different between the pre-dry treatment (n=9, mean pH 5.99 ±

335   0.05) and the pre-wet treatment (n=9, mean pH 6.17 ± 0.05) p=0.012. The soil's pH in relation

336   to days from rewetting regardless of treatment, (n=6 at each time point in days, 0, +1, +2, +3

337   and +7) was significantly different p=0.030. The interaction between days from rewetting and

338   treatment was not significantly different for pH (n=3 at each time point in days, 0, +1, +2, +3

339   and +7) p=0.537.

340   The soil's TON concentrations was not significantly different between the pre-dry treatment

341   (n=26, mean TON 0.020 ± 0.002 mg g^{-1}) and the pre-wet treatment (n=26, mean TON 0.024

342   ± 0.002 mg g^{-1}) p=0.112. The soil's TON in relation to days from rewetting regardless of

343   treatment, (n=6 at each time point in days, -14, -1, 0, +1, +2, +3 and +7) was significantly

344   different p=0.001. The interaction between days from rewetting and treatment was not

345   significantly different for TON (n=3 at each time point in days, -14. -1, 0, +1, +2, +3 and +7)

346   p=0.856. See figure 5.

347   The soil's NH_4^+ concentrations were significantly different between the pre-dry treatment

348   (n=26, mean NH_4^+ 0.036 ± 0.003 mg g^{-1}) and the pre-wet treatment (n=26, mean NH_4^+ 0.031

349   ± 0.002 mg g^{-1}) p<0.001. The soil's NH_4^+ in relation to days from rewetting regardless of

350   treatment, (n=6 at each time point in days, -14. -1, 0, +1, +2, +3 and +7) was significantly

351   different p<0.001. The interaction between days from rewetting and treatment was significantly

352   different for NH_4^+ (n=3 at each time point in days, -14, -1, 0, +1, +2, +3 and +7) p=0.009. See

353   figure 5.

354   The soil's DOC concentrations was significantly different between the pre-dry treatment (n=26,

355   mean DOC 0.297 ± 0.013 mg g^{-1}) and the pre-wet treatment (n=26, mean DOC 0.256 ± 0.015

356   mg g^{-1}) p=0.03. The soil's DOC in relation to days from rewetting regardless of treatment, (n=6

357   at each time point in days, -14, -1, 0, +1, +2, +3 and +7) was significantly different p=0.026.

358   The interaction between days from rewetting and treatment was not significantly different for

359   DOC (n=3 at each time point in days, time points -14, -1, 0, +1, +2, +3 and +7) p=0.939. See

360   figure 5.

361   **3.4 Microbiology**

362   Due to the large quantity of variables measured, the ANOVA table for DNA and RNA as well

363   a summary of the data can be found in the supplementary material. In summary, the soil's

364   DNA concentrations in terms of copy number per gram of dry soil for targeted genes

365   (Supplementary table 1) was significantly different between the pre-dry treatment and the pre-

366   wet treatment (n=26, time points in days, -14, -1, 0, +1, +2, +3 and +7) for *16S B* (p<0.026),

367   *ITS* (p<0.001) and *16S P* (p<0.007), see figure 6 and Supplementary table 2. In terms of days

368   from rewetting, (n=6 per day, time points -14, -1, 0, +1, +2, +3 and +7) the following genes

369   were significantly different: *16S A* (p<0.001)*, 16S P* (p<0.015)*, ITS* (p<0.001)*, AmoA*

370   (p<0.027), *nirS* (p<0.0390), *nirK* (p<0.017), *nosZI* (p<0.007), *nosZII* (p<0.001), *comaB*

371   (p<0.026). In terms of the interaction between treatment and days (n=3), only *ITS* was

372   significantly different (p<0.001).

373   The following gene transcripts (*AmoA, AmoB, nirS, nirK, nosZI, nosZII* and *norB)* were below

374   the level of detection under these experimental conditions despite increasing the total amount

375   of total RNA per PCR from 20 ng to 40 ng. Only Pseudomonas 16S rRNA gene transcripts, in

376   terms of copy number per gram of dry soil, was significantly different between the pre-dry

377   treatment and the pre-wet treatment (n=15, time points in days, 0, +1, +2, +3 and +7), *16S P*

378   (p=0.021) see figure 6 and Supplementary table 2. In terms of days since rewetting, (n=6 per

379   day, time points in days, 0, +1, +2, +3 and +7) the following gene transcripts were significantly

380   different: *16S B* (p=0.021), *16S A* (p=0.006), *16S P* (p<0.001), and *ComaB* (p=0.015). In terms

381   of the interaction between treatment and days (n=3), *16S A* (p=0.012) and ComaB (p<0.001)

382   were significantly different.

383

384   **4.0 Discussion**

385   This study consisted of two experiments, the first induced a range of antecedent treatments

386   to determine the relationship between the size of an N_2O hot moment, and the drought length

387   and drought intensity. The second experiment consisted of two treatments selected from the

388   first experiment to elucidate how changes in soil chemistry and microbiology are driving the

389   higher N_2O emissions post wetting. All treatments had an increase in daily N_2O emissions post

390   wetting, and all treatments besides the 30% to 60% WFPS treatment had a response that is

391   typical of a hot moment from a unfertilized soil (Priemé and Christensen, 2001, Molodovskaya

392   et al., 2012, Harrison-Kirk et al., 2013, Leitner et al., 2017, Barrat et al., 2021), wherein the

393   daily emissions post wetting are >10 times the daily emissions before rewetting, over a 24 to

394   72 hour period.

395   **4.1 Drought impact curves**

396   Drought length had a significant impact on the size of a N_2O hot moment, which is shown in

397   figures 2 and 3, which we have defined as drought impact curves. The relationship that was

398   observed was an inverted U shape, which is different to what was hypothesized (H_1), as it was

399   assumed to have an increasing positive linear response as drought intensity has been shown

400   to have this relationship (Priemé and Christensen, 2001, Ruser et al., 2006, Harrison-Kirk et

401   al., 2013). Because the size of hot moment increases and then decreases according to

402   drought length, this has significant implications for predicting the size of hot moments, as

403   future studies which will need to determine the degree of moderate drought that will induce

404   the greatest response, and define the limit for when a severe drought will rapidly reduce the

405   emissions post wetting. Moreover, while the model shows a continued curve (see figure 3), it

406   is clear from the raw data (see supplementary table 3) that as predicted (H_2) it took >6 days

407   of drying before the hot moment started to increase in size, therefore future incubation studies

408   will also need to determine the minimum drought period required before a hot moment could

409   be induced.

410   We assumed that increasing drought intensity would increase the size of the hot moment (H_3);

411   with the caveat that the final WFPS after rewetting needed to create a sufficiently anaerobic

412   soil for there to be a large increase in N_2O emissions (H_4), and this was observed, with very

413   low emissions from the 30% to 60% WFPS treatment comparative to the other treatments. It

414   is possible that this explains the results of studies like Pezzolla et al. (2019) and Owens et al.

415   (2016) that induced drying and wetting cycles, but did not observe an N_2O hot moment, as the

416   treatments never created an ideal environment for anaerobic respiration. However, in our

417   study the increase in cumulative emissions as the drought intensity was increased was only

418   significant for 40 to 90% ($p<0.05$), the other treatments that were wetted to 90% were not

419   significantly different from each other. The large difference in emissions from the 40% WFPS

420   treatment compared to the others, could be due to an important shift in the soil's matric

421   potential, as although water content is linear, matric potential is extremely non-linear, and the

422   40% WFPS treatment could be situated either side of the soils capillary fringe (Whalley et al.,

16

423   2013). Although the 50%, 60% and 70% drought curves were statistically similar, we predicate

424   that this is an artifact of the statistical and experimental approach. We therefore hypothesize

425   that if this experiment were repeated with replicates for the drought length treatments between

426   10 and 20 days, the fitted curves would be more accurate and there would be more power to

427   detect differences between wetting intensities.

4.2.1 Changes in microbiology

429   For the soil cores that were destructively sampled, we observed a decrease in the abundance

430   of most of the functional genes from DNA post wetting, which could be due to cell death as

431   the absolute change in water potential, and the rate of change over time is a stress event that

432   may cause cell death and cell lysis (Schimel, 2018). Clark and Hirsch (2008), showed that the

433   viability of culturable bacteria and extractable DNA decreased in soils that were air dried at

434   ambient conditions prior to long term archiving. This supports the decrease in functional genes

435   and viability of bacterial soil communities shown in this study under drought conditions. As

436   expected (H_5) there was no difference in the quantity of functional N cycling genes between

437   the pre-dry and pre-wet treatments, but there were two population markers that might account

438   for the differences in emissions, fungal *ITS* and pseudomonas *16S* (see figure 6). Fungal *ITS*

439   decreased less in the pre-dry soil, and it is well established that fungi are better at surviving

440   changing water potentials (Schimel, 2018, Barnard et al., 2013, Evans and Wallenstein, 2012).

441   Pseudomonas *16S* increased post wetting in the pre-dry after a slight decrease. Increases in

442   rRNA population markers could either indicate rapid growth or rapid activity, and many

443   pseudomonas are well known relatively fast growing denitrifiers (Davies et al., 1989), so this

444   population could be the source of N_2O from their growth and activity. Moreover, the delay in

445   the response of this population marker, matches the delay in the peak emissions.

4.2.2 Hot moments and the key nitrogen pathways

447   Although we hypothesised (H_7) that the transcriptional data could explain the differences in

448   emissions, the lack of differences between the two treatments in terms of mRNA and to some

449 extent DNA, suggests that the active processes are more complex than initially thought. Firstly,

450 N_2O does not seem to be produced due to an increase in anaerobic denitrification transcript

451 or DNA abundance via NO_2^- (*nir*S, *nir*K) or NO (*nor*B*)*. Secondly, it does not seem to be due

452 to a change in the rate of reduction of N_2O to N_2 (*nos*ZI, *nos*ZII). Chemodenitrification is

453 possible if there are high concentrations of NH_2OH or NO_2^- and low concentrations of O_2,

454 however the differences in TON between the treatments were not statistically significant, and

455 it is typically observed after N is artificially added (Liu et al., 2019, Liu et al., 2018, Anderson

456 and Levine, 1986). Moreover, it took several days to reach the peak N_2O emissions, whereas

457 an abiotic source would produce a peak within several hours (Leitner et al., 2017). Wang et al

458 (2017), however demonstrated that within short-term waterlogged soils, although the relative

459 abundance of denitrifiers within the soil did not significantly change, the composition of these

460 microbial denitrifiers did change and potentially to more active populations. Analysis of shifts

461 in microbial community structure were not within the scope of this study. However, an increase

462 in microbial activity, although not necessarily picked up in gene abundance, should have been

463 seen in increases in associated N-cycling transcripts, which were not observed in this study.

464 There were slight and significant differences in NH_4^+ which contrary to our hypothesis (H_6)

465 could be indicating the key process (see figure 5). In this study some form of nitrifier activity

466 seems the most probable explanation for the differences in N_2O emissions, as NH_4^+ decreased

467 rapidly in both treatments during rewetting (Leitner et al., 2017). There was no significant

468 difference between treatments for DNA or RNA in terms of *AmoA*, *AmoB*, and COMAMMOX

469 *AmoB*. Caranto and Lancaster (2017) propose that NH_4^+ is oxidised to NO via NH_2OH, which

470 can produce N_2O as a non-enzymatic by-product. This could be likely given the lack of *nirK,*

471 *nirS* or *norB* activity, and it is possible that NH_4^+ is being oxidised to NH_2OH using an, as yet,

472 undetermined gene. It is also possible that the reduction in NH_4^+ is not related to the increase

473 in N_2O, and instead it is being utilised via the ANNAMOX pathway, producing N_2. This seems

474 unlikely given that the literature has shown the important contribution NH_4^+ addition can have

475 on the emission pulse following rewetting (Slessarev et al., 2021, Leitner et al., 2017, Heil et

476 al., 2016, Zhu et al., 2013).

477 If NH_4^+ is being oxidised to NH_2OH, then further oxidation using hydroxylamine oxidoreductase

478 (*HAO*) can produce large amounts of N_2O either via NO or straight to N_2O, and this can occur

479 under anaerobic conditions (Caranto and Lancaster, 2017, Caranto et al., 2016, Otte et al.,

480 1999, Hooper and Terry, 1979). Moreover, there is increasing evidence that nitrifiers under

481 oxic shock will utilise NO_2^- and NH_2OH (Liu et al., 2019, Liu et al., 2018, Wrage-Mönnig et al.,

482 2018). Caranto et al. (2016) propose a pathway that involves the oxidation of NH_2OH which

483 produces N_2O, NO and NO_2^- as a by-product. This could be the case in this study, however

484 we did not measure *HAO* transcription levels or its genetic abundance. Besides NH_2OH

485 oxidation, there is also the possibility of nitrifier denitrification which typically involves the use

486 of NO_2^- as the electron acceptor. It is predicted that NO_2^- builds up in dry soil and therefore

487 could be readily utilised upon rewetting (Liu et al., 2018). However, we measured no changes

488 in *nirS* or *nirK* in terms of gene expression or in terms of functional gene abundance. In this

489 study the pH was significantly different between treatments, however the difference is

490 biological negligible in terms of affecting N_2O (less than 0.2) (Šlmek and Cooper, 2002). The

491 difference was likely caused by the continuous rewetting of the pre-wet samples.

492 **4.3 Future studies and the use of metabolomics**

493 Given the inconclusive evidence from this study, and the current pool of literature, we suggest

494 a new approach for future studies, which is based on the methods for exploring a similar

495 phenomena known as the Birch effect, where rewetted soils produce a pulse in CO_2 emissions.

496 The work of Warren (Warren, 2020, Warren, 2014a, Warren 2014b) has used metabolomics

497 to investigate the Birch effect, and has made significant progress in understanding how

498 osmolytes fuel the emissions pulse. This could be a relevant analogue for N_2O hot moments,

499 as the use of nitrogenous osmolytes is common in cell cultures and in soil (Schimel, 2018,

500 Warren, 2014). Studies exploring the Birch effect and osmolyte accumulation have shown that

501 it does not follow a linear response to drying, as accumulation of osmolytes is observed under

502 moderate dryness, but extreme drying conditions seems to supress this strategy (Warren,

503 2016, Kakumanu et al., 2013). This is because osmolyte accumulation is costly and at certain

504 water potentials as it no longer provides effective osmoregulation. This matches the inverted

505 U shape response in emissions observed in study. It is therefore possible in this study that the

506 time delay could indicate the mineralisation and then catabolism of osmolytes, but this will

507 need to be further investigated This study used only one soil type, and this soil was sieved

508 and had no vegetation cover, therefore future studies using different soil types with plant cover

509 might observe a different a response to changing antecedent moisture conditions.

510

511 **5.0 Conclusion**

512 In summary, this study outlined the relationship between the size of the N_2O response post

513 wetting, and the antecedent conditions of drought length and intensity. For this grassland soil,

514 there is an inverted U shaped response in terms of drought days, with 10 to 15 days of drying

515 showing the largest response, while 0 and 30 days show the smallest. We suggest a 2-stage

516 dormancy strategy to explain this, where microbes under dry conditions store osmolytes which

517 are catabolised upon rewetting, however at prolonged negative water potentials this strategy

518 is no longer effective, and so they enter a deeper state of dormancy, resulting in a dormant

519 microbial community that can no longer rapidly respond to the changing water potential. From

520 this experiment, we hypothesise that the source of the N_2O emissions is from the

521 mineralisation of osmolytes. Moreover, given the delayed response after rewetting, and the

522 inverted U shaped curve in terms of drought length, it seems likely that the majority of

523 emissions are of biological origin. Furthermore, given the lack of transcriptional activity in our

524 soil in terms of *nirK*, *nirS*, *amo* and *norB*, we suggest that pathways proposed by Caranto et

525 al. (2016) and Caranto and Lancaster (2017) seem probable where N_2O is a product of nitrifier

526 activity from the oxidation of NH_2OH.

527 Given the results of this study are from a single sieved soil type with no vegetation cover,

528 further work repeating this study's methodology will be necessary to fully elucidate the

529 relationship between drought length, wetting intensity, and the size of the N_2O hot moment. If

530 the hot moment is driven by NH_4^+ then plants using this pool could reduce the size of the hot

531 moment, especially if the soil has a more connected macro pore structure from roots and a

532 different soil texture with less clay (Ruser et al., 2006, Harrison-Kirk et al., 2013).

Data Sharing and Data Accessibility statement

543 Data from this experiment is shared in the supplementary material.

Conflict of Interest Statement

545 There are no conflicts of interest to declare.

546

References

548 ANDERSON, I. C. & LEVINE, J. S. 1986. Relative Rates of Nitric Oxide and Nitrous Oxide
549 Production by Nitrifiers, Denitrifiers, and Nitrate Respirers. *Applied and Environmental*
550 *Microbiology,* 51**,** 938-945.
551 AVERY, B. W. 1973. Soil Classification In The Soil Survey Of England And Wales. *Journal of*
552 *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,* n/a.

BARRAT, H. A., EVANS, J., CHADWICK, D. R., CLARK, I. M., LE COCQ, K. & M. CARDENAS, L. 2020. The impact of drought and rewetting on N2O 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 N2O during denitrification. *Soil Biology and Biochemistry,* 43**,** 240-250.

BRAKER, G. & TIEDJE, J. M. 2003. Nitric oxide reductase (norB) genes from pure cultures and environmental samples. *Appl Environ Microbiol,* 69**,** 3476-83.

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.

GARDES, S. M. & BRUNS, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology,* 2**,** 113-118.

GLARING, M. A., VESTER, J. K., LYLLOFF, J. E., ABU AL-SOUD, W., SØRENSEN, S. J. & STOUGAARD, P. 2015. Microbial Diversity in a Permanently Cold and Alkaline Environment in Greenland. *PLOS ONE,* 10**,** e0124863.

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.

608 HENRY, S., BAUDOIN, E., LÓPEZ-GUTIÉRREZ, J. C., MARTIN-LAURENT, F., BRAUMAN,
609   A. & PHILIPPOT, L. 2004. Quantification of denitrifying bacteria in soils by nirK gene
610   targeted real-time PCR. *J Microbiol Methods,* 59**,** 327-35.
611 HENRY, S., BRU, D., STRES, B., HALLET, S. & PHILIPPOT, L. 2006. Quantitative detection
612   of the nosZ gene, encoding nitrous oxide reductase, and comparison of the
613   abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. *Appl Environ Microbiol,*
614   72**,** 5181-9.
615 HOOPER, A. B. & TERRY, K. R. 1979. Hydroxylamine oxidoreductase of Nitrosomonas:
616   Production of nitric oxide from hydroxylamine. *Biochimica et Biophysica Acta (BBA) -*
617   *Enzymology,* 571**,** 12-20.
618 JONES, D. L. & WILLETT, V. B. 2006. Experimental evaluation of methods to quantify
619   dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil*
620   *Biology and Biochemistry,* 38**,** 991-999.
621 KAKUMANU, M. L., CANTRELL, C. L. & WILLIAMS, M. A. 2013. Microbial community
622   response to varying magnitudes of desiccation in soil: A test of the osmolyte
623   accumulation hypothesis. *Soil Biology and Biochemistry,* 57**,** 644-653.
624 LEITNER, S., HOMYAK, P. M., BLANKINSHIP, J. C., EBERWEIN, J., JENERETTE, G. D.,
625   ZECHMEISTER-BOLTENSTERN, S. & SCHIMEL, J. P. 2017. Linking NO and N2O
626   emission pulses with the mobilization of mineral and organic N upon rewetting dry soils.
627   *Soil Biology and Biochemistry,* 115**,** 461-466.
628 LIU, S., SCHLOTER, M. & BRÜGGEMANN, N. 2018. Accumulation of NO2 during periods of
629   drying stimulates soil N2O emissions during subsequent rewetting. *European Journal*
630   *of Soil Science,* 69**,** 936-946.
631 LIU, S., SCHLOTER, M., HU, R., VEREECKEN, H. & BRÜGGEMANN, N. 2019.
632   Hydroxylamine Contributes More to Abiotic N2O Production in Soils Than Nitrite.
633   *Frontiers in Environmental Science,* 7.
634 MCCLAIN, M. E., BOYER, E. W., DENT, C. L., GERGEL, S. E., GRIMM, N. B., GROFFMAN,
635   P. M., HART, S. C., HARVEY, J. W., JOHNSTON, C. A., MAYORGA, E., MCDOWELL,
636   W. H. & PINAY, G. 2003. Biogeochemical Hot Spots and Hot Moments at the Interface
637   of Terrestrial and Aquatic Ecosystems. *Ecosystems,* 6**,** 301-312.
638 MOLODOVSKAYA, M., SINGURINDY, O., RICHARDS, B. K., WARLAND, J., JOHNSON, M.
639   S. & STEENHUIS, T. S. 2012. Temporal Variability of Nitrous Oxide from Fertilized
640   Croplands: Hot Moment Analysis. *Soil Science Society of America Journal,* 76**,** 1728-
641   1740.
642 OTTE, S., SCHALK, J., KUENEN, J. G. & JETTEN, M. S. M. 1999. Hydroxylamine oxidation
643   and subsequent nitrous oxide production by the heterotrophic ammonia oxidizer
644   Alcaligenes faecalis. *Applied Microbiology and Biotechnology,* 51**,** 255-261.
645 OWENS, J., CLOUGH, T. J., LAUBACH, J., HUNT, J. E., VENTEREA, R. T. & PHILLIPS, R.
646   L. 2016. Nitrous Oxide Fluxes, Soil Oxygen, and Denitrification Potential of Urine- and
647   Non-Urine-Treated Soil under Different Irrigation Frequencies. *Journal of*
648   *Environmental Quality,* 45**,** 1169-1177.
649 PENDERGRASS, A. G. & KNUTTI, R. 2018. The Uneven Nature of Daily Precipitation and Its
650   Change. *Geophysical Research Letters,* 45**,** 11,980-11,988.
651 PEZZOLLA, D., CARDENAS, L. M., MIAN, I. A., CARSWELL, A., DONOVAN, N., DHANOA,
652   M. S. & BLACKWELL, M. S. A. 2019. Responses of carbon, nitrogen and phosphorus
653   to two consecutive drying–rewetting cycles in soils. *Journal of Plant Nutrition and Soil*
654   *Science,* 182**,** 217-228.
655 PRIEMÉ, A. & CHRISTENSEN, S. 2001. Natural perturbations, drying–wetting and freezing–
656   thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from
657   farmed organic soils. *Soil Biology and Biochemistry,* 33**,** 2083-2091.
658 RUSER, R., FLESSA, H., RUSSOW, R., SCHMIDT, G., BUEGGER, F. & MUNCH, J. C. 2006.
659   Emission of N2O, N2 and CO2 from soil fertilized with nitrate: effect of compaction, soil
660   moisture and rewetting. *Soil Biology and Biochemistry,* 38**,** 263-274.
661 SCHIMEL, J. P. 2018. Life in Dry Soils: Effects of Drought on Soil Microbial Communities and
662   Processes. *Annual Review of Ecology, Evolution, and Systematics,* 49**,** 409-432.

663 ŠIMEK, M. & COOPER, J. E. 2002. The influence of soil pH on denitrification: progress
664   towards the understanding of this interaction over the last 50 years. *European*
665   *Journal of Soil Science,* 53**,** 345-354.
666 SLESSAREV, E. W., GREENE, A. C., HOMYAK, P. M., YING, S. C. & SCHIMEL, J. P. 2021.
667   High resolution measurements reveal abiotic and biotic mechanisms of elevated nitric
668   oxide emission after wetting dry soil. *Soil Biology and Biochemistry,* 160**,** 108316.
669 THROBÄCK, I. N., ENWALL, K., JARVIS, A. & HALLIN, S. 2004. Reassessing PCR primers
670   targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with
671   DGGE. *FEMS Microbiol Ecol,* 49**,** 401-17.
672 VENTEREA, R. T., PETERSEN, S. O., DE KLEIN, C. A. M., PEDERSEN, A. R., NOBLE, A.
673   D. L., REES, R. M., GAMBLE, J. D. & PARKIN, T. B. 2020. Global Research Alliance
674   N2O chamber methodology guidelines: Flux calculations. *Journal of Environmental*
675   *Quality,* 49**,** 1141-1155.
676 VILGALYS, R. & HESTER, M. 1990. Rapid genetic identification and mapping of enzymatically
677   amplified ribosomal DNA from several Cryptococcus species. *Journal of Bacteriology,*
678   172**,** 4238-4246.
679 VSN, I. 2020. Genstart for Windows 20th Edition. VSN International, Hemel Hempstead, UK.
680 WARREN, C. R. 2014. Response of organic N monomers in a sub-alpine soil to a dry–wet
681   cycle. *Soil Biology and Biochemistry,* 77**,** 233-242.
682 WARREN, C. R. 2016. Do microbial osmolytes or extracellular depolymerisation products
683   accumulate as soil dries? *Soil Biology and Biochemistry,* 98**,** 54-63.
684 WHALLEY, W. R., OBER, E. S. & JENKINS, M. 2013. Measurement of the matric potential of
685   soil water in the rhizosphere. *Journal of Experimental Botany,* 64**,** 3951-3963.
686 WRAGE-MÖNNIG, N., HORN, M. A., WELL, R., MÜLLER, C., VELTHOF, G. & OENEMA, O.
687   2018. The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil*
688   *Biology and Biochemistry,* 123**,** A3-A16.
689 ZHU, X., BURGER, M., DOANE, T. A. & HORWATH, W. R. 2013. Ammonia oxidation
690   pathways and nitrifier denitrification are significant sources of N2O and NO under low
691   oxygen availability. *Proceedings of the National Academy of Sciences of the United*
692   *States of America,* 110**,** 6328-6333.

693

694

695

696

697

698

699

700

701

702

704

705   ***Table 1.*** *Summary characteristics of pooled soil used to pack the soil cores for the first*
706   *experiment. See figure 5 for the soil characteristics for the second experiment. Variation is*
707   *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

708

709

710

711

712

713

714

715

716

717

718

719

720

721

722

723

Experiment 1. Days indicate time spent at antecedent WFPS before being rewetted to its designated WFPS
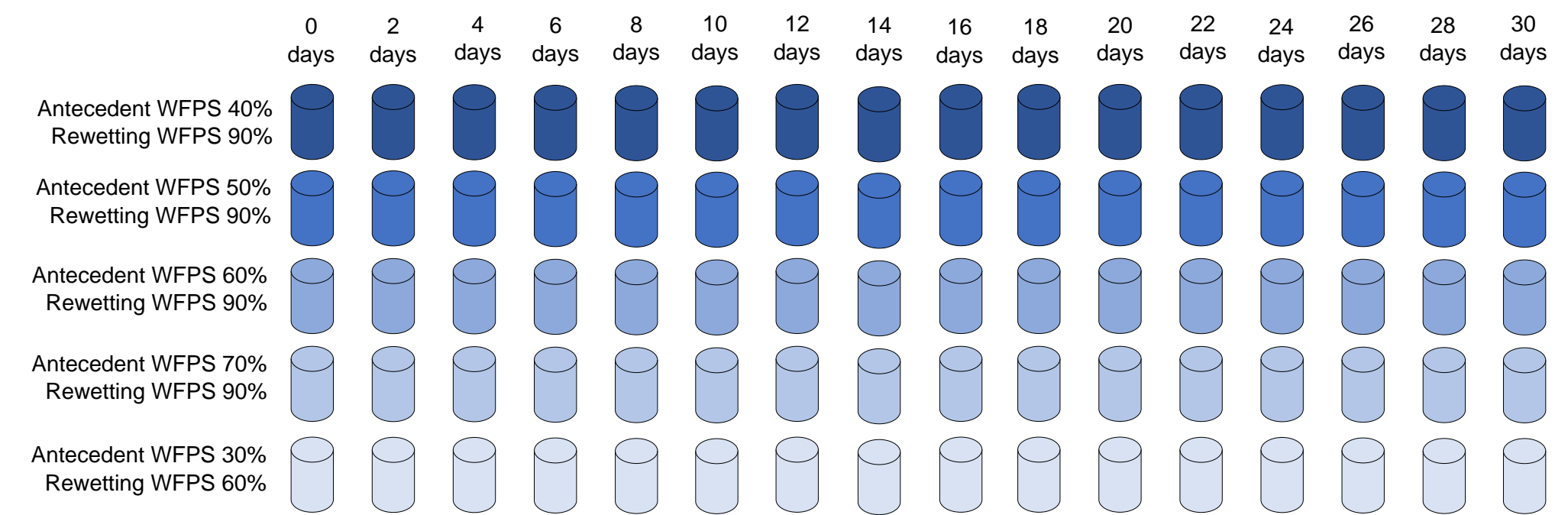
Experiment 2. Days are numbered according to when the samples were rewetted, both treatments were kept at their antecedent WFPS for 14 days
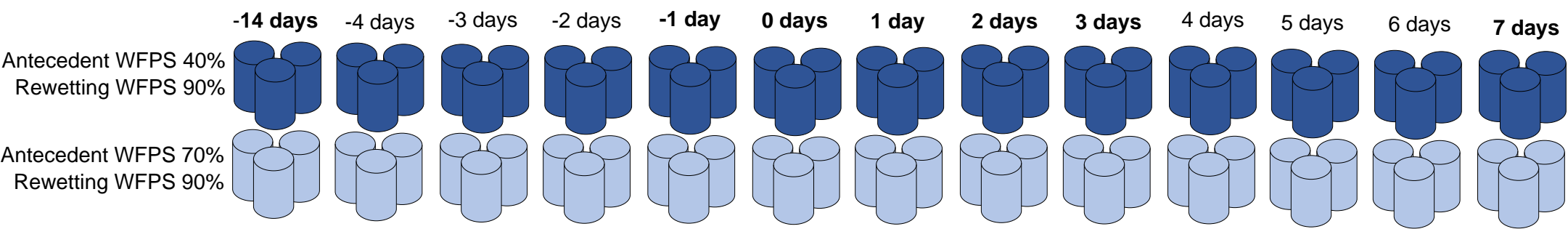
Figure 1. *Visual representation of the two experiments conducted in this study.*

725

726
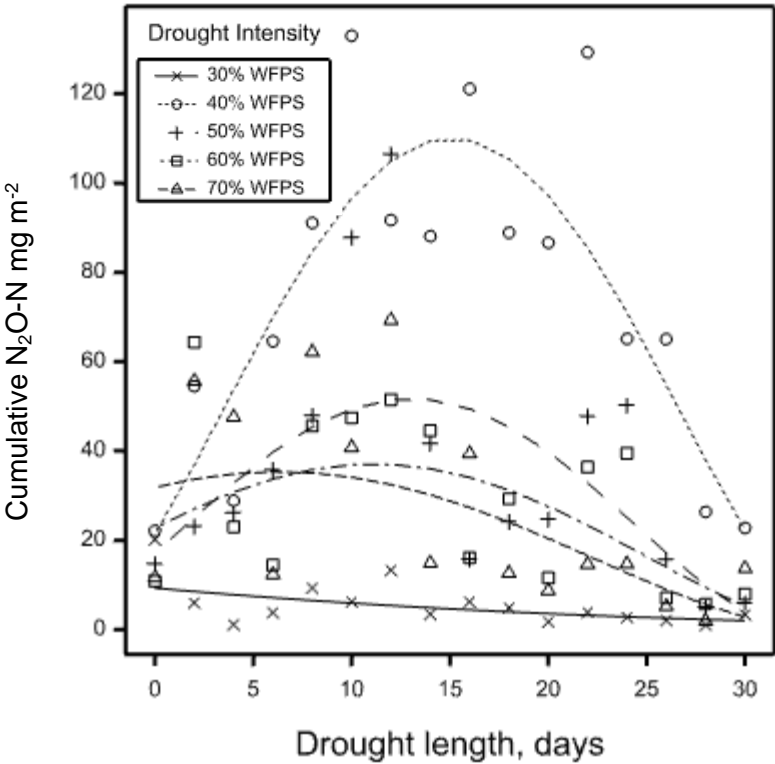
Figure 2. Scatter plot showing the relationship between the different treatments of drought intensity and drought length, and the response in cumulative N_2O-N emissions in mg m^{-2} for 14 days post wetting. All treatments were rewetted to 90% WFPS, apart from 30% WFPS which was wetted to 60%. A linear 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..

727
728
729
730
731
732

733

734

735

736

737
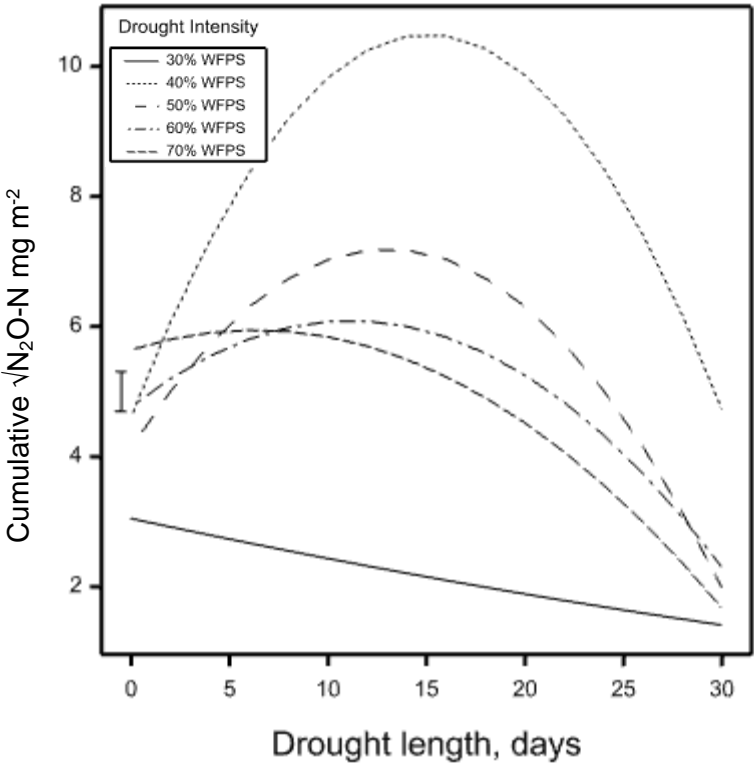
Figure 3. Linear model with quadratic term of the relationship between the different treatments of drought intensity and drought length on the X axis, and the response in square root of cumulative N_2O-N emissions mg m^{-2} 14 days post wetting. All treatments were rewetted to 90% WFPS, apart from 30% WFPS which was wetted to 60%. The error bar represents the average standard error (1.44) of the predicted responses from the model.

738

739
740
741
742
743

744

745

746

747

748

749

750
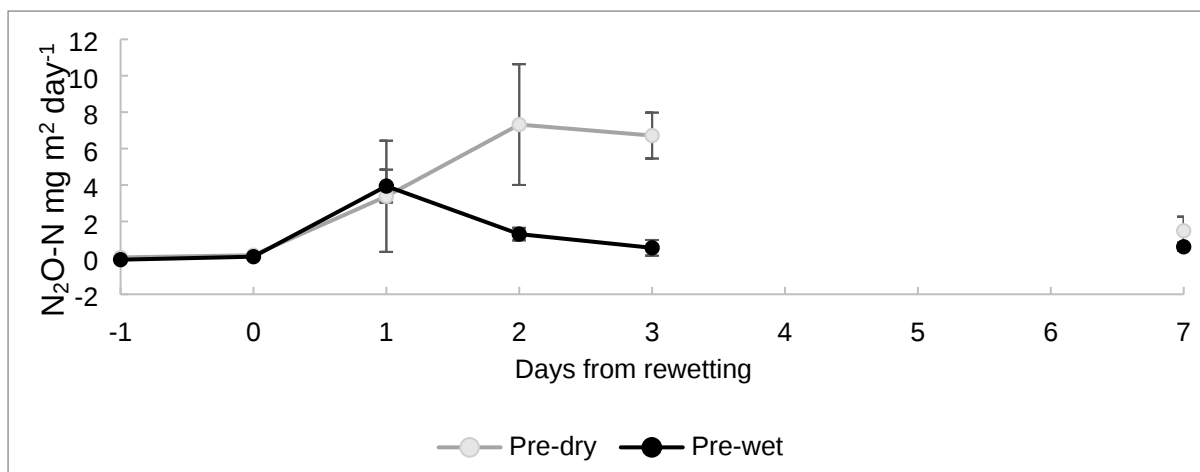
Figure 4. N_2O fluxes for the destructively sampled soil cores throughout the experiment. The pre-dry soil was kept at 40% WFPS, and wetted to 90% WFPS at day 0, pre-wet soil was kept at 70% WFPS, and wetted to 90% WFPS at day 0. Error bars represent standard error, n=3.

776

777

778
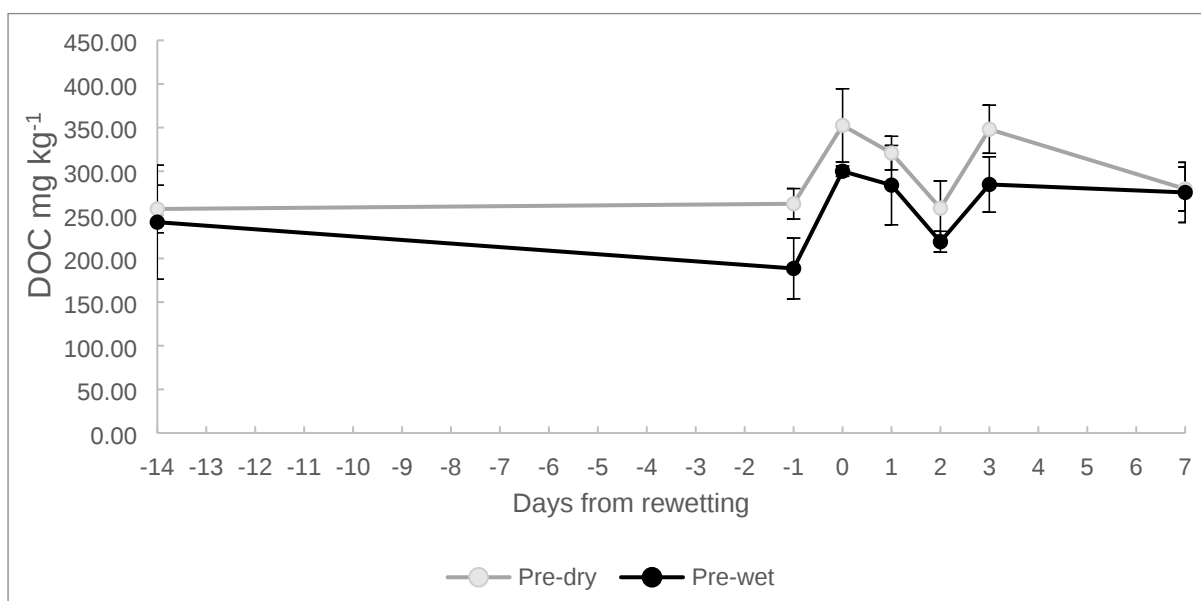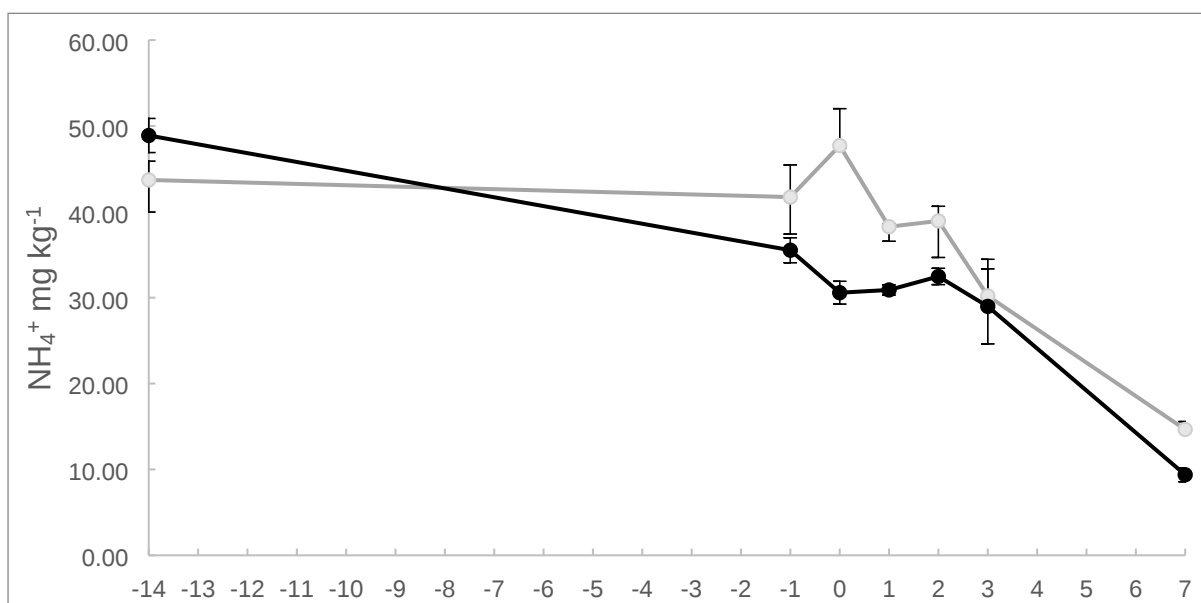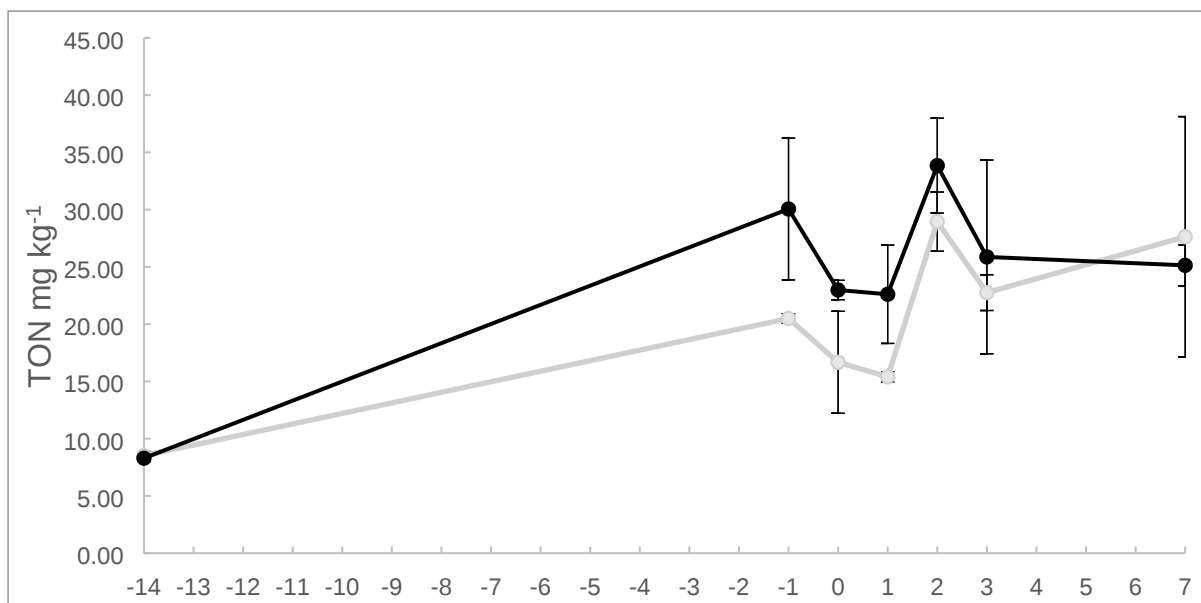
779 **Figure 5**. *Soil chemistry changes for total extractable oxidized nitrogen (TON), ammonium*
780 *(NH4+) and dissolved organic carbon (DOC) on a dry soil basis, from experiment 2. The pre-*
781 *dry soil was kept at 40% WFPS, and wetted to 90% WFPS at day 0, pre-wet soil was kept at*
782 *70% WFPS, and wetted to 90% WFPS at day 0. Error bars represent standard error, n=3 for*
783 *each treatment at each timepoint.*

784

785
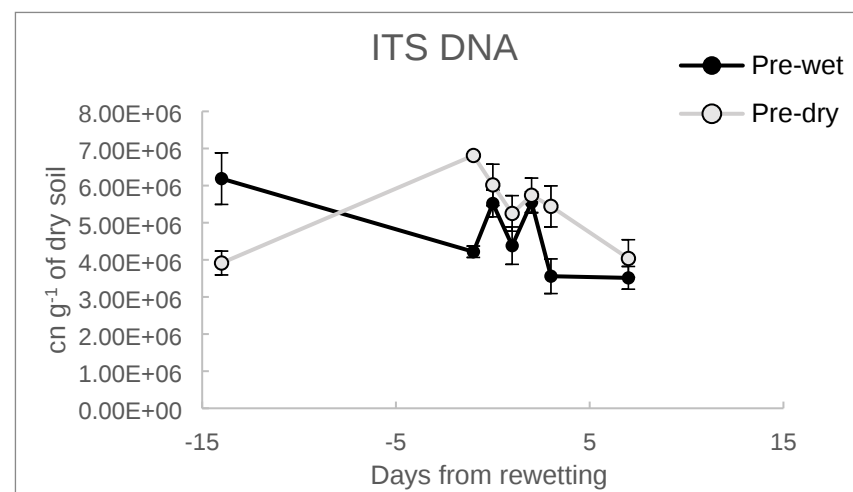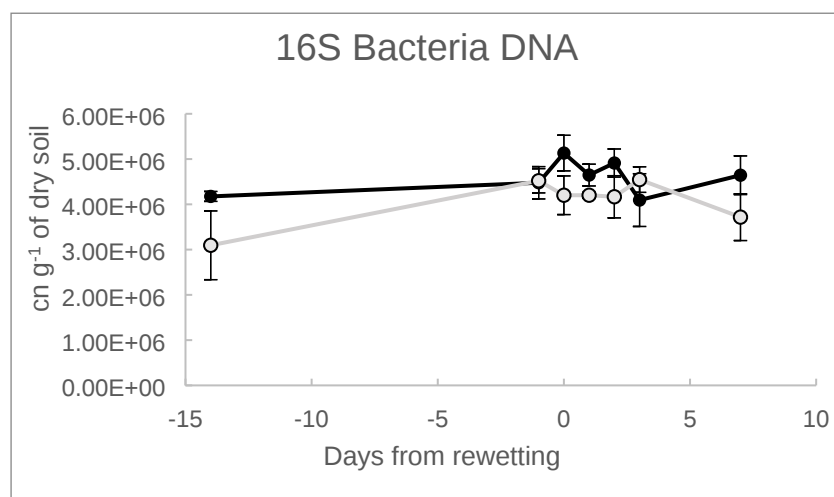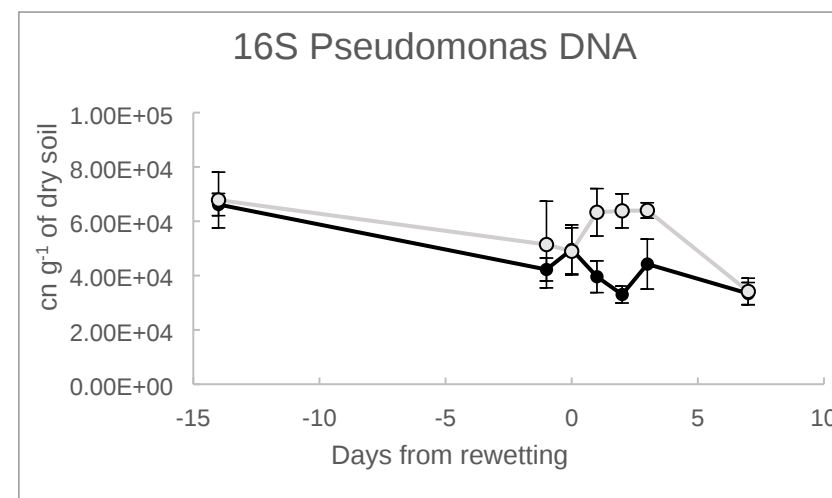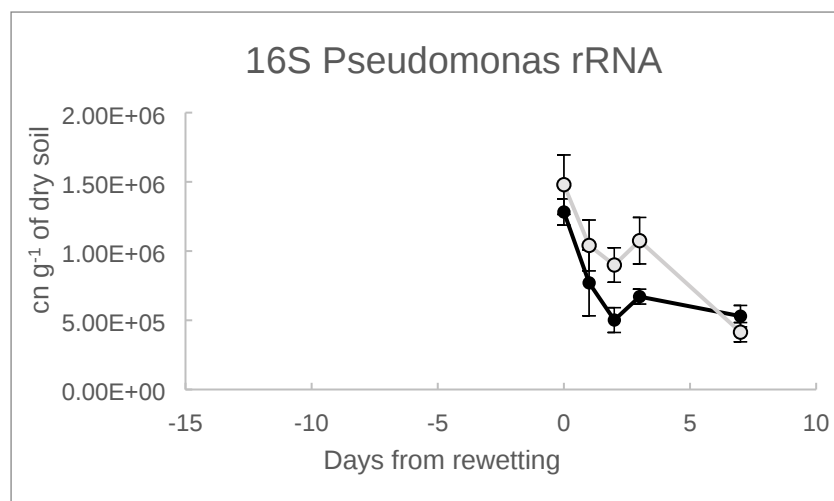
Figure 6. *Change in different functional genes throughout the experiment which were significantly different (p<0.05) according to treatment. Soil was rewetted at day 0, error bars represent standard error, n=3.*

Supplementary Tables and Figures

Supplementary Table 1. List of primers and qPCR efficiency

Target gene	DNA/RNA	Primer	Sequence 5'-3'	qPCR efficiency	References
Bacterial 16S rRNA region (16S B)	DNA and RNA	341F	CCT AYG GGR BGC ASC AG	DNA = 84.3%	Glaring et al. (2015)
		806R	GGA CTA CNN GGG TAT CTA AT	RNA = 100.9%	
Archaeal 16S rRNA region (16S A)	DNA and RNA	Parch519F	CAG CMG CCG CGG TAA	DNA = 104.7%	Øvreas 1997 Reysenbach 1995
		Arch1060R	GGC CAT GCA CCW CCT CTC	RNA = 99.5%	
Pseudomonas 16S rRNA (16S P)	DNA and RNA	16S_Pseu F1	CTT CGG GCC TTG CGC TAT CA	DNA = 103.0%	Clark and Hirsch, 2008
		16S_Pseu R1	GCCCTTCCTCCCAACTTAA	RNA = 93.6%	
Fungal Internal transcribed spacer (ITS)	DNA	ITS1f	TCC GTA GGT GAA CCT GCG G	DNA = 93.2%	Gardes and Bruns (1993) Vilgalys and Hester (1990)
		5.8s	CGC TGC GTT CTT CAT CG		
Nitrite Reductase Gene (nirK)	DNA and RNA	nirK876F	ATY GGC GGV CAY GGC GA	DNA = 115.1%	Henry et al. (2004)
		nirK1040R	GCC TCG ATC AGR TTR TGG TT	RNA = 109.0%	
Nitrite Reductase Gene	DNA and RNA	cd3aF	GTS AAC GTS AAG GAR ACS GG	DNA = 82.1%	Throbäck et al. (2004)

(nirS)		R3cdR	GAS TTC GGR TGS GTC TTG A	RNA = 81.5%	Hallin et al. 1999
Nitrous Oxide Reductase Clade I (nosZI)	DNA and RNA	nosZ1F	CGC RAC GGC AAS AAG GTS MSS GT	DNA = 85.4%	Henry et al. (2006)
		nosZ1R	CAK RTG CAK SGC RTG GCA GAA	RNA = 113.3%	
Nitrous Oxide Reductase Clade II (nosZII)	DNA and RNA	nosZIIF_11 62-1178	CTI GGI CCI YTK CAY AC	DNA = 88.0%	Jones et al. 2013
		nosZIIR_1 889 -1907	GCI GAR CAR AAI TCB GTR C	RNA = 84.4%	
Nitric oxide reductase subunit B (norB)	DNA and RNA	qnorB5R-F	TGG TGG GTN GTN CAY CTN TGG GT	DNA = 83.5%	Braker and Tiedje (2003)
		qnorB7R	GGN GGR TTD ATC ADG AAN CC	RNA =112.3%	
ammonia monooxygenase Archaeal (AmoA)	DNA and RNA	arch-amoAF	STA ATG GTC TGG CTT AGA CG	DNA = 83.7%	Francis et al 2005
		arch-amoAR	GCG GCC ATC CAT CTG TAT GT	RNA = 83.2%	
ammonia monooxygenase Bacterial (AmoB)	DNA and RNA	amoA-1F	GGG GTT TCT ACT GGT GGT	DNA = 101.2%	Rotthauwe et al (1997)
		amoA-2R	CCC CTC KGS AAA GCC TTC TTC	RNA = 99.6%	

Comammox bacteria amoA (ComaB)	DNA and RNA	comaB-244F comaB-659R	TAY TTC TGG ACR TTY TA ARA TCC ARA CDG TGT G	DNA = 95.7%	Pjevac et al 2017

790

Supplementary Table 2. *p values from ANOVA for the functional genes for DNA and RNA. Treatment represents the pre-dry or the pre-wet treatment, and days the day the soil cores were sampled. For the experimental design see section 2, results where p<0.05 are in bold.*

Source of variation	16S B	16S A	16S P	ITS	amo A	amo B	nirS	nirK	nosZ 1	nosZ 2	coma B	norB
Treatment	**0.026**	0.097	**0.007**	**0.015**	0.910	0.124	0.631	0.936	0.521	0.851	0.153	0.105
Days	0.258	**0.001**	**0.015**	**0.001**	**0.027**	0.138	**0.039**	**0.017**	**0.007**	**0.001**	**0.026**	0.768
Treat.Days	0.465	0.635	0.281	**0.001**	0.281	0.487	0.587	0.095	0.059	0.310	0.373	0.096

795

Source of variation	16S B	16S A	16S P	Coma B	amo A
Treatment	0.959	0.755	**0.021**	0.084	0.403
Days	**0.021**	**0.006**	**0.001**	**0.015**	0.353
Treat.Days	0.158	**0.012**	0.398	**0.001**	0.536

796

797

798

799

800

801

802

803

804

805 **Supplementary Table 3.** *Summary of the daily N_2O-N emissions data organized according to the timeline of rewetting in mg m^{-2} per day. At day*
806 *0 samples were rewetted to 90% waterfilled pore space (WFPS) apart from the 30% treatment which was rewetted to 60%. Cumulative emissions*
807 *are calculated from day 0 to day 14. The missing data at the very beginning are due to constraints on experimental design, as all soil cores had*
808 *to be packed and wetted on the same day.*

Treatment	WFPS	Drying days	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Cumulative N_2O mg/m^2
40W 0	40	0		-0.2	6.0	2.3	3.6	2.9	2.1	1.4	0.6	0.6	0.4	0.6	0.7	0.6	0.4	22.1
40W 2	40	2	1.2	17.7	13.0	6.2	4.7	2.9	1.6	1.6	0.7	1.1	1.1	1.1	0.5	0.7	0.3	54.5
40W 4	40	4	2.1	12.1	3.8	2.2	1.0	1.0	0.9	0.8	0.6	0.5	0.7	0.6	0.7	1.0	1.0	28.9
40W 6	40	6	0.0	15.5	11.7	4.7	5.7	4.4	4.2	3.5	1.5	2.5	2.8	2.3	2.0	2.3	1.7	64.5
40W 8	40	8	0.0	22.5	24.0	11.9	6.4	4.7	3.4	3.0	2.9	2.4	2.3	2.1	1.9	1.7	1.7	91.1
40W 10	40	10	10.8	35.4	24.7	18.8	10.0	6.4	4.0	3.7	2.9	2.6	3.0	2.6	2.9	3.0	2.1	133.0
40W 12	40	12	1.0	18.5	30.6	14.3	8.0	4.0	2.9	2.5	1.7	2.2	1.2	1.4	1.0	1.5	1.1	91.7
40W 14	40	14	1.2	18.0	23.7	12.2	6.5	5.1	2.1	3.4	3.3	2.5	2.5	2.2	2.0	2.0	1.4	88.1
40W 16	40	16	0.3	18.3	25.7	24.0	9.9	8.9	6.4	4.5	4.3	3.7	2.7	2.9	3.2	3.3	3.0	121.1
40W 18	40	18	0.3	15.4	15.2	14.7	8.1	7.0	5.2	5.4	3.9	3.0	2.3	2.2	1.8	2.3	2.1	88.9
40W 20	40	20	0.5	7.9	19.0	12.4	11.6	9.9	4.6	4.4	2.6	2.5	1.8	2.1	4.0	1.8	1.4	86.6
40W 22	40	22	0.3	5.9	21.2	27.8	19.0	13.1	11.2	6.2	6.1	5.4	2.1	3.8	4.0	4.1	-0.9	129.3
40W 24	40	24	0.2	17.0	13.4	13.4	5.1	3.6	3.1	2.8	2.0	1.4	1.5	1.2	-0.7	1.1	0.3	65.1
40W 26	40	26	0.7	5.6	15.1	14.9	11.4	6.7	3.0	1.8	1.8	1.5	1.1	0.7	0.5	0.2	0.1	65.0
40W 28	40	28	0.1	4.1	8.1	7.8	2.5	1.5	0.8	0.6	-0.1	0.4	0.1	-0.1	-0.1	0.5	0.2	26.4
40W 30	40	30	0.2	5.4	9.5	2.7	1.8	0.9	-0.1	0.5	0.3	0.3	-0.1	0.2	0.1	0.2	0.7	22.7
50W 0	50	0		0.6	2.5	1.1	3.0	3.1	1.9	0.8	0.2	0.1	0.8	0.0	0.3	-0.1	0.3	14.7
50W 2	50	2	0.6	4.4	9.3	3.6	1.6	1.0	0.4	0.2	0.5	0.2	0.4	0.2	0.3	0.1	0.3	23.1

50W 4	50	4	1.4	12.4	7.1	1.9	0.5	0.6	0.3	0.4	0.4	0.1	0.3	0.3	0.0	0.4	0.1	26.2
50W 6	50	6	0.2	6.5	6.8	6.1	4.7	3.5	3.5	2.2	0.4	0.3	0.5	0.1	0.3	0.3	0.3	35.8
50W 8	50	8	0.1	10.6	12.8	6.5	4.6	2.8	2.2	1.9	1.3	1.0	1.1	1.0	0.7	0.7	0.6	48.0
50W 10	50	10	1.3	10.2	21.2	14.1	14.2	8.4	7.0	3.5	1.9	1.9	1.3	1.1	0.9	0.7	0.1	87.8
50W 12	50	12	0.6	4.7	20.5	18.5	12.8	11.5	7.5	7.6	4.9	4.6	3.2	3.5	2.4	2.3	1.8	106.5
50W 14	50	14	0.8	12.3	11.4	6.1	2.8	2.0	1.9	1.4	0.8	0.4	0.4	0.5	0.6	0.1	0.2	41.7
50W 16	50	16	0.5	2.0	3.5	4.0	0.7	1.3	0.3	0.9	0.5	0.6	0.5	0.2	0.4	0.0	0.4	15.8
50W 18	50	18	0.4	3.9	4.9	6.5	3.1	2.2	1.1	0.1	0.8	0.2	0.2	0.5	0.0	0.2	0.1	24.3
50W 20	50	20	0.5	8.7	5.4	2.9	1.3	1.5	1.3	0.7	0.5	0.4	0.3	0.4	0.1	0.3	0.3	24.7
50W 22	50	22	0.3	3.6	9.1	8.6	5.5	4.1	3.1	3.6	2.4	1.8	1.4	1.5	1.4	1.1	0.4	47.8
50W 24	50	24	0.3	11.7	14.2	7.3	4.1	3.1	2.5	2.4	1.4	0.7	0.5	0.5	0.3	0.5	0.6	50.3
50W 26	50	26	0.4	1.6	5.0	2.7	1.8	1.5	1.3	0.4	0.4	0.2	0.0	0.1	0.3	0.1	0.1	15.7
50W 28	50	28	0.5	0.8	1.0	0.7	0.3	0.2	0.2	0.1	0.2	0.3	0.1	0.1	0.2	0.0	0.3	5.0
50W 30	50	30	0.4	1.1	2.8	0.5	0.5	0.1	0.1	0.2	0.3	0.1	0.2	0.0	0.1	0.0	-0.3	5.9
60W 0	60	0		0.2	2.0	2.3	0.9	2.4	1.4	0.4	0.2	0.2	0.3	0.2	0.0	0.0	0.3	10.8
60W 2	60	2	0.1	3.0	27.8	22.3	5.7	1.5	0.9	0.8	0.5	0.4	0.3	0.4	0.1	0.4	0.1	64.3
60W 4	60	4	4.6	14.6	2.4	0.5	0.2	0.2	0.2	0.0	0.1	-0.1	-0.1	0.2	-0.2	0.3	0.1	23.0
60W 6	60	6	0.2	3.1	2.9	2.3	1.7	1.4	0.4	0.7	0.4	0.4	0.3	0.2	0.2	0.2	0.1	14.4
60W 8	60	8	0.2	6.2	15.4	6.5	5.0	3.2	2.3	1.9	1.4	1.4	1.0	0.1	0.4	0.4	0.2	45.6
60W 10	60	10	1.5	4.1	7.0	6.2	6.5	6.1	4.5	3.6	2.0	1.9	1.5	1.0	0.7	0.3	0.6	47.4
60W 12	60	12	0.7	5.1	11.9	8.1	5.5	4.6	3.4	3.0	2.1	2.1	1.8	1.2	0.7	0.7	0.6	51.5
60W 14	60	14	0.4	6.5	7.1	5.0	4.5	3.7	2.4	2.8	2.5	2.1	2.0	1.5	1.7	1.1	1.3	44.5
60W 16	60	16	0.5	2.5	4.9	3.3	1.1	1.1	0.3	0.5	0.5	0.6	0.1	0.0	0.2	0.0	0.5	16.1
60W 18	60	18	0.7	2.7	5.6	3.7	2.9	2.5	1.9	1.7	1.4	1.5	1.2	0.8	0.9	0.9	1.0	29.3
60W 20	60	20	0.2	2.4	3.9	1.5	1.8	0.5	0.4	0.2	0.1	0.1	0.2	-0.2	0.1	0.1	0.2	11.6
60W 22	60	22	0.3	1.9	4.1	6.3	4.0	3.0	2.6	3.7	2.3	2.4	1.9	1.0	1.4	1.7	-0.3	36.4

60W 24	60	24	0.2	4.9	7.1	5.1	3.8	2.9	3.1	3.1	2.4	1.4	2.2	1.2	-0.1	1.5	0.7	39.4
60W 26	60	26	0.4	0.7	2.0	1.6	1.3	0.5	0.3	0.2	0.1	0.1	0.1	0.0	0.1	0.0	-0.4	7.1
60W 28	60	28	0.2	0.3	1.9	1.6	0.3	0.5	0.3	0.2	0.7	-0.1	-0.1	0.0	-0.1	0.1	-0.1	5.7
60W 30	60	30	0.2	1.2	3.0	0.6	0.7	0.5	0.6	0.5	0.4	0.3	0.2	0.1	-0.3	0.0	-0.1	7.9
70W 0	70	0		0.2	0.7	1.5	2.0	3.0	1.2	0.4	0.3	0.3	0.6	0.1	0.5	0.6	0.3	11.6
70W 2	70	2	-0.1	1.7	29.6	16.4	4.1	0.5	1.2	0.2	0.3	0.3	0.3	0.5	0.1	0.2	0.4	55.6
70W 4	70	4	2.9	13.4	3.1	7.1	8.2	7.5	4.7	-0.7	0.0	0.1	0.2	0.0	0.4	0.3	0.5	47.6
70W 6	70	6	0.4	2.7	2.0	2.1	1.2	0.5	0.8	0.7	0.3	0.4	0.4	0.1	0.3	0.3	0.1	12.3
70W 8	70	8	0.2	16.5	16.2	9.2	5.4	4.5	2.8	2.0	1.7	1.0	0.6	0.6	0.4	0.4	0.7	62.2
70W 10	70	10	0.4	10.6	14.0	6.6	3.5	1.1	1.2	0.7	0.6	0.5	0.6	0.4	0.3	0.2	0.1	40.8
70W 12	70	12	0.3	3.1	10.7	12.4	10.8	10.0	5.5	4.5	2.0	2.9	1.8	1.9	1.2	0.9	1.3	69.3
70W 14	70	14	0.6	2.4	3.2	2.0	1.6	1.0	0.9	0.4	0.5	0.5	0.5	0.4	0.5	0.1	0.3	14.9
70W 16	70	16	0.3	6.6	10.9	7.1	4.0	2.5	2.0	1.8	0.9	1.1	0.6	0.5	0.5	0.4	0.3	39.4
70W 18	70	18	0.4	1.8	3.3	2.4	1.5	1.0	0.6	0.4	0.5	0.0	0.3	0.1	0.1	0.3	-0.1	12.6
70W 20	70	20	0.6	2.3	2.0	1.8	0.7	0.5	0.2	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.0	8.7
70W 22	70	22	0.2	0.7	1.7	3.9	2.6	1.0	0.9	0.9	0.5	0.6	0.3	0.6	0.3	0.3	0.1	14.6
70W 24	70	24	0.2	2.2	2.0	2.1	1.0	0.7	0.9	1.0	0.6	1.0	0.8	0.9	0.5	0.4	0.4	14.7
70W 26	70	26	0.1	0.7	1.7	1.1	0.7	0.3	0.1	0.3	0.2	0.1	-0.2	0.1	0.0	0.0	0.0	5.2
70W 28	70	28	0.2	0.2	0.5	0.6	0.3	-0.1	0.2	0.0	-0.2	0.1	0.0	0.2	0.0	-0.2	0.2	1.9
70W 30	70	30	0.3	1.7	2.0	2.2	2.4	3.2	-0.1	0.8	0.2	0.4	0.2	0.2	0.0	0.0	0.0	13.7
30W 0	30	0		4.8	0.1	8.8	0.2	1.1	0.5	0.9	0.4	1.7	0.9	0.7	0.1	0.1	0.0	20.2
30W 2	30	2	0.0	2.7	0.7	0.6	0.0	0.4	0.4	1.1	0.1	0.2	0.0	0.1	-0.1	0.0	-0.2	6.0
30W 4	30	4	0.5	0.4	0.2	-0.1	0.1	0.4	-0.3	0.1	-0.2	0.0	0.1	0.0	0.3	-0.1	-0.2	1.1
30W 6	30	6	0.1	1.0	0.7	0.5	0.5	0.4	0.1	0.1	0.1	0.0	0.1	0.2	0.0	0.2	-0.1	3.8
30W 8	30	8	0.0	1.6	5.2	0.3	0.3	0.6	0.4	0.1	-0.1	0.3	-0.1	0.1	0.0	0.1	0.3	9.3
30W 10	30	10	3.2	1.2	-1.2	1.2	0.2	0.6	0.4	0.1	0.2	0.0	0.0	0.1	0.1	0.2	0.0	6.2

30W 12	30	12	0.2	0.6	2.2	1.0	1.5	1.9	0.9	0.5	1.6	0.4	0.4	0.3	0.6	0.9	0.2	13.3
30W 14	30	14	0.4	0.0	0.6	0.5	0.2	0.5	0.1	0.0	0.5	0.3	-0.1	0.2	0.1	0.1	0.1	3.4
30W 16	30	16	0.1	0.7	1.1	1.1	1.0	0.6	0.5	0.5	0.3	0.1	0.4	-0.2	0.2	0.1	-0.1	6.2
30W 18	30	18	0.4	0.5	0.9	1.2	0.6	0.1	0.1	0.3	0.2	0.3	0.3	-0.2	0.0	0.1	0.1	4.9
30W 20	30	20	0.0	1.1	0.0	0.1	0.1	0.2	0.1	-0.3	-0.1	0.0	0.1	0.0	0.2	0.1	0.1	1.7
30W 22	30	22	0.1	0.2	0.1	0.6	0.7	0.2	0.2	0.5	0.2	0.1	0.0	0.3	0.3	0.1	0.2	3.9
30W 24	30	24	0.0	0.2	0.3	0.5	0.2	0.2	0.2	0.0	0.0	-0.1	0.3	0.2	0.2	0.4	0.2	2.8
30W 26	30	26	0.1	0.3	0.6	0.3	0.2	0.2	0.2	-0.1	-0.1	0.1	0.1	-0.1	0.1	0.0	0.0	2.1
30W 28	30	28	0.4	0.5	0.2	0.2	0.0	0.0	0.1	0.1	0.0	0.1	0.0	-0.1	-0.2	0.0	-0.2	1.0
30W 30	30	30	0.3	0.3	0.7	0.2	0.5	0.4	0.8	0.1	0.3	-0.1	-0.1	-0.1	0.0	-0.1	0.1	3.3

809

810

811 **Supplementary Table 4.** Soil's DNA concentrations in terms of copy number per gram of dry soil for targeted genes at all time points and for all
812 genes measured. Note each value is the average of two technical replications.

Treatment	Days	16S B	16S A	16S P	ITS	amoA A	amoA B	nirS	nirK	nosZ1	nosZ2	comaB	norB
DRY	-14	1784901	15213	50091	3563943	843	25702	73476	1177728	453696	36096	28401	396322
DRY	-14	3086858	19699	67741	3623093	1248	37342	69560	2170493	529175	29668	37230	427477
DRY	-14	4414264	20025	85627	4554669	5179	57832	84404	2641583	702323	44351	41766	475296
WET	-14	4044463	18216	73115	4826604	2898	29737	77302	2294419	587535	33659	29624	481754
WET	-14	4393270	24154	66360	6618094	2479	51139	86013	3064599	700898	45967	28248	470956
WET	-14	4086467	24157	59003	7118583	643	40491	79740	2650701	615231	35880	40172	437492
DRY	-1	4001264	15696	22830	6760213	1175	22149	91865	2377426	671346	36746	50922	508361
DRY	-1	4667355	15871	77950	6781580	882	15489	80581	2142421	597781	42878	38313	577981
DRY	-1	4894141	19968	53494	6892751	865	18232	68968	2064644	568369	50020	41882	828643
WET	-1	5007531	17773	35151	4354224	621	23887	75457	2240103	644280	47088	66382	563895
WET	-1	4625241	13812	41810	4386913	688	12417	69106	2061634	579726	50097	34945	472681
WET	-1	3797320	9939	49749	3910948	642	15632	64539	1237918	458553	32820	37456	422741
DRY	0	3508596	23902	39357	5419557	2792	31447	89892	1509287	566893	72989	38209	440171

|													
DRY	0	4114253	32334	66253	5506822	1345	75085	106732	2906160	650365	79354	42099	420137
DRY	0	4979860	33596	41224	7129164	829	37473	105451	3541823	767734	83021	31088	526759
WET	0	5414526	33010	66406	4965137	1582	55300	94547	2715349	732179	108699	39107	627148
WET	0	5627095	31167	35378	5399803	769	50989	94299	3340629	799182	85799	48130	603988
WET	0	4355305	21908	47119	6191729	571	38579	86355	2396510	653091	53651	39489	474490
DRY	1	4206560	23974	45994	6123398	3566	45283	91536	2881283	714399	60116	32265	369492
DRY	1	4309206	27815	74157	4462579	2293	48857	81143	2763729	718174	65725	32215	507355
DRY	1	4088490	22918	69748	5163210	635	38751	72097	2401519	557242	49026	33961	463168
WET	1	4952772	25469	49170	4877996	8671	52588	85247	2406630	515876	78427	52406	641478
WET	1	4815154	18461	40561	4893280	3537	46345	70970	1905866	598341	80676	35456	598410
WET	1	4172365	16918	29016	3372412	1530	19995	53660	1464213	427976	63304	42067	451680
DRY	2	3290970	13520	73084	4985604	719	39810	93495	1245889	477160	51420	38109	435742
DRY	2	4328641	28456	51846	5652367	1539	35482	85644	1704985	566935	84405	42132	737792
DRY	2	4882450	25323	66571	6580875	1287	115803	68019	1847083	610009	93350	41232	605415
WET	2	4542921	17181	27168	5109117	764	27238	68982	2014799	554349	57808	39476	441855
WET	2	5529851	20367	34064	5458622	967	17343	70786	2154542	540882	65347	45420	540745
WET	2	4664696	14827	37808	6082014	638	29563	75212	1942043	572214	29059	44017	490536
DRY	3	5056097	17759	58310	6455913	3049	27841	68559	2401483	643081	58830	38931	493202
DRY	3	4079121	11064	67058	5314861	1856	86035	61428	1741834	559863	40495	27306	407459
DRY	3	4504911	17015	66641	4551045	838	43853	14660	2000519	519696	48936	47940	441570
WET	3	5260948	17353	62258	3939169	1638	53655	16333	1790842	530835	68708	65734	568088
WET	3	3536184	11032	32112	2629976	1484	21399	91510	1286111	348622	62506	30871	429156
WET	3	3485890	6000	38321	4106878	919	20954	104748	1383318	442837	32071	44742	326973
DRY	7	2737687	9117	31845	3880466	1340	23279	88097	2053959	448261	36831	25221	328022
DRY	7	3918570	14562	43488	4983716	1090	35355	76503	2292037	541730	35914	34003	450463
DRY	7	4496261	17344	27124	3244165	422	31267	106701	2160465	450351	36122	57861	440073
WET	7	4214795	10554	41446	2903770	818	50230	85868	2427466	503241	34288	57407	419662
WET	7	4218129	10793	30235	3885084	447	21829	88865	2132967	562092	30501	74432	478147
WET	7	5496686	13519	28473	3751851	473	16984	84471	2871051	616882	57083	56680	509752

813

815

816

817

818

819

820

821

822

Supplementary Table 5. Soil's RNA concentrations in terms of copy number per gram of dry soil for targeted genes at all time points and for all genes measured. Asterisks indicate that the values were below the detection limit. Note each value is the average of two technical replications.

Treatment	Days	16S B	16S A	16S P	ComaB	amoA A	amoA B	nirS	nirK	nosZ1	nosZ2	norB
DRY	0	1988912	545410	1059367	268	54	*	*	*	*	*	*
DRY	0	3558211	666555	1611159	369	71	*	*	*	*	*	*
DRY	0	2915687	883931	1768205	262	56	*	*	*	*	*	*
WET	0	4227282	718031	1174863	427	82	*	*	*	*	*	*
WET	0	2509437	677337	1200387	323	61	*	*	*	*	*	*
WET	0	3851802	838456	1471698	357	52	*	*	*	*	*	*
DRY	1	3165826	706376	1404953	578	82	*	*	*	*	*	*
DRY	1	2902801	645183	898440	431	62	*	*	*	*	*	*
DRY	1	2387945	621896	818603	289	52	*	*	*	*	*	*
WET	1	1644035	410859	1237296	316	50	*	*	*	*	*	*
WET	1	1765836	426783	457745	295	54	*	*	*	*	*	*
WET	1	2198467	399261	615899	246	36	*	*	*	*	*	*
DRY	2	2193817	624746	1147200	451	53	*	*	*	*	*	*

DRY	2	1603940	674097	747589	349	41	*	*	*	*	*	*
DRY	2	2657877	853500	803127	363	54	*	*	*	*	*	*
WET	2	1962898	366290	323628	237	49	*	*	*	*	*	*
WET	2	1911199	698482	611302	483	36	*	*	*	*	*	*
WET	2	2593658	787131	570329	260	177	*	*	*	*	*	*
DRY	3	2492200	336779	939800	346	33	*	*	*	*	*	*
DRY	3	2514277	653201	1408729	458	42	*	*	*	*	*	*
DRY	3	1520075	380356	876446	228	29	*	*	*	*	*	*
WET	3	2371121	434275	661706	344	32	*	*	*	*	*	*
WET	3	2110379	459885	582531	350	33	*	*	*	*	*	*
WET	3	1895928	470140	768095	422	51	*	*	*	*	*	*
DRY	7	1823971	465900	484603	269	31	*	*	*	*	*	*
DRY	7	2553710	519119	482887	281	32	*	*	*	*	*	*
DRY	7	3008431	684372	275166	384	60	*	*	*	*	*	*
WET	7	2810610	701629	477601	624	39	*	*	*	*	*	*
WET	7	2959198	1127445	681934	853	87	*	*	*	*	*	*
WET	7	2319891	985835	430002	696	44	*	*	*	*	*	*

825

826

827
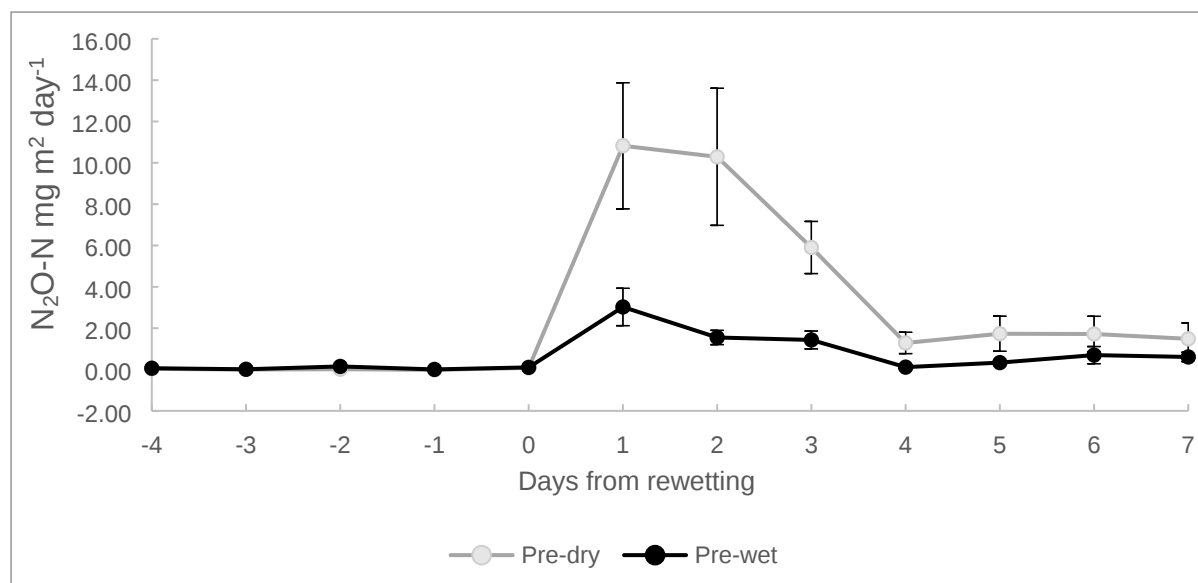
Supplementary Figure 1. *Average daily N_2O-N fluxes for all subsamples throughout the experiment. The pre-dry soil was kept at 40% WFPS, and wetted to 90% WFPS at day 0, pre-wet soil was kept at 70% WFPS, and wetted to 90% WFPS at day 0. Error bars represent standard error, varied replicate number each time point, see the methods section for details.*

831