Flooding-induced N₂O emission bursts controlled by pH and nitrate in agricultural soils

Hansen, Mette; Clough, Tim J.; Elberling, Bo

Published in:
Soil Biology & Biochemistry

DOI:
[10.1016/j.soilbio.2013.10.031](https://doi.org/10.1016/j.soilbio.2013.10.031)

Publication date:
2014

Document version
Peer reviewed version

Citation for published version (APA):
Hansen, M., Clough, T. J., & Elberling, B. (2014). Flooding-induced N₂O emission bursts controlled by pH and nitrate in agricultural soils. *Soil Biology & Biochemistry*, 69, 17-24. <https://doi.org/10.1016/j.soilbio.2013.10.031>

Flooding-induced N₂O emission bursts controlled by pH and nitrate in agricultural soils

Mette Hansen^a, Tim J. Clough^b & Bo Elberling^{c*}

^aDepartment of Geosciences and Natural Resource Management, University of Copenhagen,
Øster Voldgade 10, DK-1350 Copenhagen K., Denmark, metha@geo.ku.dk

^bAgriculture & Life Sciences Division, PO Box 84, Lincoln University, Canterbury, New
Zealand, timothy.clough@lincoln.ac.nz

^cCenter for Permafrost (CENPERM), Department of Geosciences and Natural Resource
Management, University of Copenhagen, Øster Voldgade 10, DK-1350 Copenhagen K.,
Denmark, be@geo.ku.dk,

* Corresponding author: be@geo.ku.dk, 0045 35322520

*Keywords: N₂O, flooding, denitrification, dissimilatory NO₃⁻ reduction to ammonium
(DNRA), agriculture, microsensor, stabile isotope*

Abstract

Agricultural soils are a major source of the greenhouse gas nitrous oxide (N_2O) to the atmosphere. Increasing frequency and severity of flooding as predicted for large intensively cropped areas may promote temporary denitrification and N_2O production but the effect of flooding events on N_2O emissions is poorly studied for agricultural systems. The overall N_2O dynamics during flooding of an agricultural soil and the effect of pH and NO_3^- concentration has been investigated based on a combination of the use of microsensors, stable isotope techniques, KCl extractions and modelling. This study shows that non-steady state peak N_2O emission events during flooding might potentially be at least in the order of reported annual mean N_2O emissions, which typically do not include flood induced N_2O emissions, and that more than one-third of the produced N_2O in the soil is not emitted but consumed within the soil. The magnitude of the emissions are, not surprisingly, positively correlated with the soil NO_3^- concentration but also negatively correlated with liming (neutral pH). The redox potential of the soil is found to influence N_2O accumulation as the production and consumption of N_2O occurs in narrow redox windows where the redox range levels are negatively correlated with the pH. This study highlights the potential importance of N_2O bursts associated with flooding and infers that annual N_2O emission estimates for tilled agricultural soils that are temporarily flooded will be underestimated. Furthermore, this study shows that subsurface N_2O reduction is a key process limiting N_2O emission and that a reduction in N_2O emissions is achievable if highly fertilized N-rich soils are limed.

1. Introduction

Future climate change will lead to changes in precipitation worldwide. A higher frequency of extreme rainfall events is predicted for temperate areas such as New Zealand and Northern Europe (IPCC, 2007; Min et al., 2011). This increases the risk of flooding for low-lying or poorly drained areas, which are the same areas receiving run off and ground water with potentially high nitrate (NO_3^-) concentrations. As a consequence it can be expected that there will be an increase in nitrous oxide (N_2O) production and emissions from these areas, particularly fertilized and nitrogen-rich agricultural fields (Knowles, 1982).

Nitrous oxide is a greenhouse gas with a global warming potential relative to CO_2 of 298 on a 100 year time horizon assuming a lifetime of 114 years in the atmosphere (IPCC, 2007). Additionally, N_2O has a negative effect on stratospheric ozone as NO and other free radical species (NO_x), generated from N_2O , deplete the ozone layer (Badr & Probert, 1993). The atmospheric concentration of N_2O has increased since pre-industrial times by 16% from 270 ppb to 319 ppb in 2005 (IPCC, 2007) and it is currently considered the dominant anthropogenic ozone depleting substance emitted (Ravishanakara et al., 2009). Soils are the main source of both anthropogenically and naturally produced N_2O and changes in land use have been the primary driver for the observed increase in tropospheric N_2O concentration (IPCC, 2007). Today, agricultural fields account for 42% of the total anthropogenic contribution of N_2O to the atmosphere and N_2O is the single most important greenhouse gas when looking at agricultural soils (IPCC, 2007).

In oxygen (O_2) limited environments production of N_2O in soil occurs as microbial processes utilize nitrogenous compounds as electron acceptors (Knowles, 1982). During denitrification N_2O is an obligate intermediary product in the reduction of NO_3^- to N_2 , a

process performed by heterotrophic microorganisms. It is also a by-product during dissimilatory NO_3^- reduction (DNRA) to ammonium (NH_4^+) as NO_3^- is reduced to NH_4^+ via nitrite (NO_2^-) by fermentative microorganisms (Tiedje et al., 1982). Denitrification rates increase with organic C and NO_3^- availability, soil water content, pH and temperature (Knowles, 1982; Šimek and Cooper, 2002). The $\text{N}_2\text{O}:\text{N}_2$ ratio, describing the end product of denitrification, shifts in favour of N_2O as soil NO_3^- concentrations and acidity increase (Knowles, 1982; Weier et al., 1993). Not all N_2O produced in a soil will be emitted as it can be consumed during denitrification to N_2 a process controlled by the presence of N_2O reductase (NOS) (Knowles, 1982). Highly anoxic conditions, caused by high soil water content and high availability of easily degradable organic matter, favour the consumption of N_2O (Wrage et al., 2001) as NOS is strongly inhibited by the presence of O_2 (Knowles, 1982). Thus the balance between N_2O consumption and production rates controls N_2O emissions as well as the transport properties of N_2O in the soil (Clough et al., 2005). The primary mode of transport for N_2O in the soil is diffusion, which is controlled by concentration gradients according to Fick's law of diffusion.

The environmental factors for production of N_2O are optimal when fertilized fields are flooded. Non-steady state draining experiments have established the relationship between water-filled pore space and N_2O emissions (Castellano et al., 2010), however, to the authors knowledge, no studies on agricultural soils and only a few studies on natural soils have examined the effect of soil flooding on N_2O dynamics: the temporal and spatial trends of subsurface N_2O concentrations and net surface emissions. Jørgensen and Elberling (2012) found a distinct pulse pattern in N_2O concentrations and emissions when flooding an unmanaged wetland peat soil. An increase in N_2O concentrations was observed within the first 24 hrs followed by a rapid decline in concentration, until the N_2O concentration was below

detection after 40 hrs. It was concluded that for these wetland peat soils the increase in N₂O production would not affect the annual N₂O emission budget, even if flooding event frequency increases in the future (Jørgensen and Elberling, 2012). This may not be the case for agricultural fields, where tillage events can increase the availability of NO₃⁻-N (Eriksen and Jensen, 2001; Silgram and Shepherd, 1999) and thereby the potential for N₂O production via denitrification.

The aim of this study was to investigate the overall N₂O dynamics during a flooding event of a New Zealand agricultural soil as affected by soil pH and NO₃⁻ concentration. Specific aims of the study were to determine the balance between produced, consumed and emitted N₂O from the soil and to determine the depth- and time-specific production and consumption of N₂O. Two methods were used in combination to achieve the aims: depth-specific profiling of the soil N₂O concentration and the redox potential using microsensors as well as 2 M KCl extractions of 3 soil layers per soil core after ¹⁵N labelled NO₃⁻ addition. The study was designed based on the hypothesis that it is possible to mitigate N₂O emissions by changes in agricultural practises (with a focus on changes in soil pH and N-input) and that annual N₂O inventories made to date have potentially been underestimated because the impact of flooding has not been included in annual budgets.

2. Materials and methods

A Templeton silt loam soil (Udic Ustochrept) was collected from a field, with a management history of perennial pasture, from the top layer (0-10 cm deep) during cultivation for pasture renovation, Lincoln, Canterbury (43° 38.720S; 172° 26.753E Lat/Lon). The Canterbury region is temperate with mean annual precipitation of 600-700 mm and a daily mean air temperature range of 1-10°C in the coldest months and 12-22°C in the warmest (Cappelen and Jensen, 2001). The Templeton soil and similar inceptisols represents app. 25% of the Canterbury Plains (Molloy, 1988). Inceptisols in temperate areas are soils with high inputs of fertilizer N (Potter et al., 2010) with crop types typically consisting of cereals such as barley and wheat (Leff et al., 2004).

The sampled soil was air-dried, sieved (< 2 mm) and kept dry and cold (4°C). The soil pH was determined (10 g air-dried soil:25 mL water). Half of the soil was treated with 2.08 g $\text{Ca}(\text{OH})_2 \text{ kg}^{-1}$ dry soil (quicklime) in powder form to increase the pH by one unit. Lime treatment and the resulting pH increase were made consistently with previous experiments (Clough et al., 2003). Inorganic-N and dissolved organic carbon (DOC) were determined for both the un-treated and the limed soil. Inorganic-N was determined in a 2 M KCl extraction (4 g soil:70 mL KCl, shaken on an end-over-end shaker for 60 min and filtered through Whatman 42 filter paper. Filtered samples were analysed using an Alpkem FS3000 twin channel flow injection analyser (FIA) with Alpkem Winflow 4.03 software). The DOC was analysed by a DI water extraction (1:10 soil:water ratio), shaken on an end-over-end shaker for 30 min followed by centrifuging at 3500 rev/min for 20 min and filtered through a Whatman 42 filter paper into a 30 mL sample vial (Ghani et al., 2003). The DOC was determined based on the difference between the total organic carbon (TOC) and the inorganic

carbon (IC) analysed using a Shimadzu Total Organic Carbon Analyser (TOC-5000A) fitted with a Shimadzu ASI-5000A autosampler.

2.1 Core preparations

Soil was packed into either stainless steel metal cores ($D = 7.4$ cm) for microsensor measurements or PVC plastic cores ($D = 7.5$ cm) for KCl extractions (see below). Soil core bases were covered with a 1 mm nylon mesh and packed to a depth of 3.5 cm. The soil was packed in layers to ensure an even bulk density of 1 g cm^{-3} throughout the profile. Four treatments were made: control (soil with no additions, **TC**), limed soil (soil plus lime, **TL**), soil with N added (soil plus nitrate- ^{15}N , **TN**), and soil with N and lime added (soil plus nitrate- ^{15}N and lime, **TLN**). For treatment TN and TLN a known volume of ^{15}N enriched (50 atom%) KNO_3 solution (0.0154 M) was sprayed onto a designated mass of dry soil prior to packing the soil cores, supplying $100 \mu\text{g NO}_3\text{-N g}^{-1}$ soil. Since NO_3^- is evenly distributed in the surface of a cultivated soil, the $^{15}\text{N-NO}_3^-$ was applied to the entire depth of soil in the packed soil core. Soil cores were packed and adjusted with KNO_3 immediately before flooding. The soil cores were then flooded from below immediately prior to commencement of microsensor measurements, to mimic the rise of a high groundwater table, by placing them in a water-filled box. This method of soil flooding also minimised soil drainage during wetting. In total 108 cores were made, of which 12 were used for microsensor measurements and 96 for KCl extractions. KCl extractions were performed on three replicates at eight time steps for each treatment. The timing of the KCl extraction was distributed throughout the pulse of N_2O production (see supporting information (SI) (Fig. S1)). Due to measurement constraints of the microsensor, replication was done in time by sequentially measuring one soil core from one treatment at any given time. For each treatment a total of 3 replicates were measured at t_1 , t_2 and t_3 . In practice, two replicates of each treatment (at t_1 and t_2) were

measured after each other. All t_1 and t_2 measurements for all four treatments were finished within 50 days. All t_3 measurements were subsequently measured after this period (see SI Fig. S2 for exact specifications of the timing of t_1 , t_2 and t_3 for all treatments). Each soil core was followed until no more N_2O evolved (up till 7 days) before the next soil core was measured, hence only one soil core for microsensor measurements was flooded at a time. Soil remained sieved but unpacked at 4°C and unamended with ^{15}N , with these procedures performed prior to microsensor measurements starting.

2.2 Microsensors

A standard N_2O microsensor (N_2O -100, Unisense, Science Park, DK-8000 Aarhus, Denmark), a redox microsensor (RD-100, Unisense) and a redox reference electrode (REF-RM, Unisense) were used to measure the N_2O concentration and the redox potential from the soil:water interface, down through the soil profile at 500 μm steps (71 points per profile), with movement controlled by a motorized micromanipulator. To ensure a complete mixing of the water phase above the soil, an ambient air flow was generated over the water surface to avoid N_2O accumulation in the water overlying the soil core. To ensure the soil water remained at a constant level at all times, water was added several times daily to offset any evaporation, maintaining a 1 cm water depth above the soil surface. The output current for the N_2O microsensor was measured using a Microsensor Multimeter while the redox signal was measured using a pH/mV-METER. The N_2O microsensor was calibrated with a five-point calibration using a saturated N_2O solution to make standard solutions increasing by 100 μM each step. Repeated calibration, after profiling, revealed that instrument drift was insignificant. Each soil core profile was measured every two hours for 7 days or until the N_2O concentration was below the detection limit ($< 0.1 \mu M$) at all depths. The room temperature during the microsensor measurements was in the range of 21-23°C. Based on the microsensor

measurements, contour maps of the subsurface N₂O concentration and the redox potential were constructed using kriging interpolation (Surfer Version 9.785, Golden Software Inc., Colorado, USA).

2.2.1 Flux determination

The observed flux of N₂O from the flooded soil core was determined as the diffusive gas exchange across the diffusive boundary layer (DBL) according to Elberling and Damgaard (2001). The DBL is a thin film of water at the soil:water interface (often < 2 mm) where the only form of transport is molecular diffusion (Gundersen and Jørgensen, 1990). The linear concentration gradient over the DBL was used to determine the N₂O flux across the DBL using Fick's law (Clough et al., 2005). The diffusion coefficient for N₂O in water at 20°C was taken to be $2.2295 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Ramsing and Gundersen, 2009). Verification of the flux was made based on dark, closed chambers (INNOVA 1313, LumaSense, Inc., Ballerup, Denmark), where the observed flux was compared to chamber measurements performed immediately after the microsensor measurements (see SI Fig. S3).

2.2.2 Modelling N₂O production and consumption

Assuming the N₂O concentration profile represents a pseudo-steady state, the SensorTrace PRO 3.0 programme (Unisense A/S, Science Park, DK-8000 Aarhus, Denmark) was used to model the depth-specific N₂O production and consumption zones. The programme is based on the model PROFIL by Berg et al. (1998). Here the effective gas diffusion coefficient through the soil is described as a function of the water-filled pore space, the soil porosity and the associated gas diffusion coefficient through air (Berg et al., 1998).

2.3 KCl extractions

Based on the preliminary results of the maximum N₂O concentrations in the soil core, measured using the microsensors, 8 time steps for the KCl extractions were chosen for each treatment distributed over time: One extraction was made at time zero (and subsequently used as a reference for the initial conditions), three were made between time zero and maximum N₂O concentration where a further extraction was made, and then two further extractions taken after the maximum N₂O concentration and the final extraction was taken when N₂O was no longer measurable (for details, see SI Fig. S1).

Prior to KCl extraction, the flooded cores were removed to drain for app. 10 minutes. A subsample of the soil, 10 g, from the top (0-1.2 cm) of the core was mixed with 60 mL 2 M KCl in a 107 mL glass bottle capped with an aluminium screw-top lined with a rubber septum leaving a headspace of 43 mL and vigorously shaken (30 seconds). Gas samples (8 mL) were then collected from the headspace, using a gas-tight syringe fitted with a stop-cock to prevent under-pressurisation of the sample, and stored in a pre-evacuated 6 mL Exetainer®. For all ¹⁵N-NO₃⁻ treated samples an additional 16 mL gas sample was collected in a similar manner and placed in a pre-evacuated 12 mL Exetainer®. The soil/KCl solution was then shaken for 1 hour on an end-over-end shaker, left to settle for 5 minutes and filtered through Whatman 42 filter paper into a 30 mL sample vial and frozen (-20°C) until analysis. The KCl extraction was repeated with a soil sample from the middle of the soil core (1.2-2.3 cm) and from the bottom (2.3-3.5 cm). The remainder of the soil at each depth was weighed and dried to determine the gravimetric water content.

Gas samples in the 6 mL Exetainers were analysed for methane (CH₄) and N₂O using a SRI 8610C gas chromatograph (SRI, Ca. USA) linked to a Gilson 222XL autosampler. CH₄ was determined using a flame ionisation detector (FID) while N₂O was determined with an electron capture detector (ECD) calibrated with certified gas standards (BOC gases) that

covered the range of sample concentrations (Mosier and Mack, 1980). The gas samples in the 12 mL Exetainers were analysed for $^{15}\text{N-N}_2\text{O}$ and $^{15}\text{N-N}_2$ using a continuous flow isotope ratio mass spectrometer (IRMS) on a Sercon 20-20 IRMS with ^{15}N calculations performed according to the equations of Mulvaney and Boast (1986) and Stevens et al. (1993). When calculating the release of ^{15}N labelled gas entrapped in the soil, corrections were made to allow for headspace gas dissolved in the KCl solution using the appropriate Bunsen coefficient (Moraghan and Buresh, 1977). Derived ^{15}N data permitted the transformation and redistribution of the original $^{15}\text{NO}_3^-$ to be observed across inorganic-N and gaseous N species at the times KCl extractions were performed.

Soil KCl extracts were thawed at room temperature and analysed for NH_4^+ -N and NO_3^- -N concentrations using an Alpchem FS3000 twin channel flow injection analyser (FIA) with Alpchem Winflow 4.03 software. Following the method of Brooks et al. (1989) sub-samples of the KCl extracts were diffused and analysed to determine their NH_4^+ - ^{15}N and NO_3^- - ^{15}N enrichments.

2.4 Statistical analyses

Statistical analyses of Pearson product-moment correlation, F-test and one-way ANOVA analyses were performed using the SPSS statistical package (v. 19.0), with a significance level of at least 95 % (* $P < 0.05$). For KCl extractions three replicates were analysed ($n = 3$) while for microsensor N_2O determinations two replicates were analysed ($n = 2$), t_1 and t_2 .

3. Results

The initial values of the soil pH, NO_3^- concentration and DOC concentration as well as the effect of liming on the parameters can be seen in Table 1.

3.1 N_2O dynamics

For all microsensor measurements the repeated measurements at t_1 and t_2 can be seen as replicates because of the low variation (see SI Fig. S4) whereas t_3 differs markedly. For all treatments, the maximum N_2O concentrations were lower and peaked in half the time compared with t_1 and t_2 , and at much lower levels. In the following, results will be based on t_1 and t_2 , (see SI Fig. S4 for comments on t_3).

Regardless of treatment the N_2O concentrations initially increased and then decreased over time but with differences in the timing of the maximum concentration (26-34 hrs) and the maximum magnitude of the N_2O concentration (Fig. 1 and SI Fig. S5). There was a significant correlation between the observed N_2O diffusion flux and the maximum N_2O concentration in the soil ($r = 0.94$, $n = 256$, $***P < 0.0001$) (Fig. 1a). Both parameters had a skewed bell-shaped pattern with a steep increase followed by a steep decrease after which they levelled out app. 72 hrs after flooding. The highest N_2O concentrations occurred in treatment TN, followed by TL, TC and TLN. The maximum N_2O diffusion flux was $2.5 \cdot 10^{-11}$, $3.8 \cdot 10^{-11}$, $5.7 \cdot 10^{-11}$ and $2.9 \cdot 10^{-11}$ mol N_2O cm^{-2} s^{-1} for treatments TC, TL, TN and TLN, respectively. N_2O fluxes obtained using microsensor measurements are in agreement with levels obtained by INNOVA chambers (SI Fig. S3). During the first 96 hrs of flooding, the mean ($\pm$ stdev, $n = 2$) integrated N_2O fluxes for treatments TC, TL, TN and TLN were 29 ± 4.3 , 41 ± 0.9 , 54 ± 5.2 and 39 ± 8.9 mmol N_2O m^{-2} respectively.

After the initial short lag phase of app. 5 hrs the N_2O concentrations increased primarily in the zone 1-2 cm below the soil surface (Fig. 1b). Concentrations of N_2O below 2 cm depth decreased faster than in shallower depths and reached zero within 24-50 hrs after flooding (Fig. 1b and SI Fig. S5). After the maximum N_2O concentrations in the soil profile occurred, they then decreased with time in all depths still with the highest concentrations in the middle zone of the soil profile, until the N_2O concentrations were zero at all depths.

The decrease in NO_3^- concentrations correlated negatively with increasing N_2O concentrations (Fig. 1d) within the first 24-36 hrs (three time steps) for all four treatments. Subsequently, the rate of NO_3^- decrease reached a minimum over the last three time steps. For treatment TLN, the rate difference in NO_3^- decrease was small between the first and the last three time steps and the NO_3^- concentration did not reach zero during the experiment. Despite this, the N_2O concentrations were below detection for treatment TLN after 96 hrs.

As a consequence of flooding, the soil redox potential decreased over time with the rate of decrease increasing with increasing soil depth (Fig. 1c and SI Fig. S5). This depth driven change in redox potential happened primarily after the first 16 hrs of flooding. The primary redox range, where N_2O accumulates in the soil, for each of the treatments can be seen in Table 1. High N_2O concentrations were found in the same range for treatments TC, TN and TLN while the redox range for treatment TL was 100 mV lower. The N_2O concentrations and the redox potentials were significantly (* $P < 0.05$) correlated (Table 1), as the N_2O concentrations portray a bell-shaped curve over time with a decreasing redox potential where a 'build-up' and a 'build-down' phase are divided around the occurrence of the maximum N_2O concentration (see SI Fig. S6).

3.2 N_2O production and consumption

The depth-specific consumption and production rates over time were modelled using the SensorTrace PRO 3.0 programme for each treatment, while assuming that the porosity and the effective diffusion coefficient were uniform throughout the packed soil core. The diffusion flux was simultaneously determined for each profile. In Fig. 2, 6 time intervals of the depth-specific activity are shown for treatments TC and TN. The corresponding figure for TL and TLN can be seen in SI (Fig. S7). The production (positive activity rates) of N₂O commenced after 10 hrs, primarily right below the DBL. The activity was several orders of magnitude larger for treatment TN compared to TC. After 20 hrs of flooding, N₂O was produced in the near-surface zone (2 cm) of the soil while consumption of N₂O (negative activity rates) increased below this depth. Thirty hrs after flooding, high production and maximum consumption rates were found, with consumption in the top (< 0.5 cm) and below 1.5 cm and production in a 1 cm zone from 0.5 to 1.5 cm below the surface. This pattern remained throughout the experiment, with rates of production and consumption decreasing with time. The model was successfully validated as a significant correlation, with a slope of 0.9, was found between the observed flux and the modelled flux ($r = 0.77$, $n = 754$, $***P < 0.0001$).

Based on the time-integrated modelled diffusion flux and the time and depth-integrated N₂O production, more than one-third of N₂O produced in the soil was consumed within the soil and not released. Consumption accounted for 41 ± 6.9 , 34 ± 3.7 , 51 ± 0.4 and $48 \pm 10.9\%$ of N₂O produced for treatments TC, TL, TN and TLN, respectively.

3.3 ¹⁵N recovery

Percentage recovery of ¹⁵N as NO₃⁻, N₂O, N₂ and NH₄⁺ over time for treatments TN and TLN can be seen in Fig. 3. The atom% ¹⁵N enrichment for N₂O, NO₃⁻ and NH₄⁺ as well as ¹⁵X_N for N₂ (the mole fraction of ¹⁵N in the N pool from which the N₂ was derived) are shown in SI

310 (Fig. S8). At time 0 all added ^{15}N was present as NO_3^- . Over time recovery of ^{15}N in the NO_3^- -
311 ^{15}N labelled pool is reduced while the other components increase. ^{15}N recovered as N_2O
312 increased for the first 6 time steps (38 and 48 hrs after flooding for treatments TN and TLN
313 respectively) after which it decreased to zero at 96 and 144 hrs after flooding. ^{15}N recovered
314 as NH_4^+ increased steadily over the entire flooding period and ended up constituting 1.0 and
315 0.6% of the ^{15}N initially added for treatments TN and TLN, respectively. ^{15}N recovered as N_2
316 also increased over time. For treatment TN the increase was slow for the first 38 hrs,
317 subsequently it increased rapidly to $115 \pm 12\%$ ^{15}N recovered at 72 hrs and levels out at $108 \pm$
318 13% after 96 hrs within the same range of the standard deviation. For treatment TLN the ^{15}N
319 recovery of N_2 was close to zero within the first 72 hrs. After 144 hrs of flooding $^{15}\text{N}\text{-N}_2$
320 constituted 26% of the initially added ^{15}N label. The recovered $^{15}\text{N}_2$ is displayed without
321 standard deviations as less replicates are available as some fluxes were too low to be detected
322 with only one replicate available in some cases. This is primarily the case for treatment TLN,
323 and results should only be seen as best estimates.

4. Discussion

The soil profile N₂O concentrations measured using the microsensor are in the same range as the methodologically comparable study by Jørgensen and Elberling (2012), as the maximum N₂O concentration for TC is a factor 1.5 higher and the maximum N₂O diffusion flux is a factor of 4 higher than their measurements on a flooded temperate peat soil. However, the duration of the high N₂O emissions is longer when compared to other studies, resulting in an accumulated N₂O release (29-54 mmol N₂O m⁻² for the four treatments) that is at least a factor of 10 higher: For the study by Jørgensen and Elberling (2012), the duration was 40 hrs, resulting in an accumulated N₂O release of 0.06 mmol N₂O m⁻², while an experimentally flooded natural tropical wetland soil resulted in peak emissions of 2.92 and 3.7 mmol N₂O m⁻² for a 2.3 and 3 day peak duration (Liengaard et al., 2013).

There is a lack of studies examining peak N₂O emissions during flooding events from temperate agricultural fields. Choudhary et al. (2001) found the annual N₂O emission for a conventionally grown maize field in New Zealand to be 8.5-12.2 mmol N₂O m⁻² yr⁻¹ while Roelandt et al. (2005), summarizing the data from 30 studies recording the annual N₂O emissions from croplands and grasslands in North America and Europe, found emissions varied from 0.7 to 20.7 mmol N₂O m⁻² yr⁻¹. These reported values are of the same order or smaller than the peak emissions found in this study. Thus, the non-steady state emissions, over just a single four day flooding period, reported here, can potentially contribute more N₂O to the atmosphere than the annual emissions of N₂O on croplands and grasslands. This emphasizes the importance of incorporating flooding events in studies of annual N₂O emissions and the need for further in-situ measurement of N₂O fluxes during flood events.

4.1 Treatment effects

During flooding redox conditions and N_2O concentrations were markedly affected. The pH of the soil is also likely to have increased in all treatments following flooding due to reduction processes such as Fe and Mn oxide reduction (Yu and Patrick, 2003) and denitrification (Zárate-Valdez et al., 2006). Thus pH effects on N_2O emission are therefore a consequence of both the direct effect of liming and the indirect effects of redox processes.

The redox potential in the soil was a time- and depth-specific parameter during flooding (Fig. 1c). The fact that the reduction was faster in the bottom part of the soil relative to the top is in line with the fact that reduction in the top will be counter-balanced by diffusion of atmospheric O_2 into the soil core. A lower redox potential at a higher pH is in agreement with the negative correlation between the two parameters described by Yu & Patrick (2003). The range in values of these parameters is likewise in agreement with their findings.

During flooding, the development of the subsurface N_2O concentration over time portrays the same bell-shaped profile and the same depth-specific distribution, independent of treatment, also described by Liengaard et al. (2013). Higher NO_3^- concentrations resulted in higher N_2O concentrations and emissions, except in treatment TLN. The reduced N_2O production due to higher soil pH was significant when comparing TN and TLN, as expected (Šimek & Cooper 2002), whereas this effect was not seen between TC and TL.

The unambiguous depth-specific distribution of the N_2O concentration can be explained by the correlation between N_2O concentration and redox potential based on the distinct redox ranges for N_2O accumulation and reduction. The low N_2O concentration in the top soil can be explained by the combination of redox potentials being too high for N_2O accumulation and by the diffusion of N_2O to the atmosphere. The level of reduction in the middle part of the soil displayed the optimum redox potential where the denitrification process is promoted, but not

to an extent where N_2O was rapidly reduced to N_2 . The low N_2O concentrations found in the bottom part of the soil was either due to the fact that the low redox potentials favoured complete denitrification, not allowing N_2O to accumulate, or that the denitrification process was completely inhibited, allowing NO_3^- to be preserved (as seen for treatment TLN), where any measured N_2O was a result of diffusion of N_2O produced in the middle zone. Subsurface accumulation of N_2O and resulting emissions are therefore time-dependent as the redox potential of flooded soils will continuously decrease.

The depth-specific distribution of the N_2O concentrations and the finding that distinct zones of production and consumption of N_2O occur underlines the fact that spatial and temporal changes in denitrification rates are not a sequential process, but rather a consequence of micro zones of specific environmental conditions affecting the N_2O dynamics. More than one-third of the produced N_2O in the soil was consumed within the soil, with the highest ratios for treatments TN and TLN with high NO_3^- concentrations. The high N_2O production rate observed for treatment TN was counterbalanced by high consumption rates. The consumption fraction of N_2O is low when compared to the study by Liengaard et al. (2013) where about two-thirds of the produced N_2O was consumed within the soil. The balance between produced, consumed and emitted N_2O is therefore not a universal value, but a soil and environment dependent value.

The net effect of flooding, liming and addition of N was reduced N_2O emissions, as treatment TLN had the lowest emission during the time of study. The high NO_3^- concentration of treatment TLN and the lower maximum N_2O concentration indicate that the denitrification process was limited even at the NO_3^- reducing step. The lack of NO_3^- reduction explains the higher redox potential, as the soil was not exhausted of easily available electron acceptors. The limitation was not caused by the addition of ^{15}N as the incomplete reduction of $^{15}\text{N}\text{-NO}_3^-$

was of the same magnitude as the total NO_3^- reduction over time, dismissing isotope fractionation as the cause. Additionally, Pan et al. (2012) did not find NO_3^- reduction to be sensitive to pH variations within the pH range of the present study. Contrary to the other treatments, no CH_4 production was seen for TLN (see SI Fig. S9) despite the fact that a higher pH should increase the production (Le Mer and Roger, 2001). Thus, the net effect of the treatment on the gas producing processes remains uncertain.

Small concentrations of CH_4 were produced during flooding (see SI Fig. S9). Compared to N_2O , CH_4 is not a significant greenhouse gas to consider during short term floodings as the redox potential in the soil is not fully reduced to levels (< -100 mV) where CH_4 is the primary end product of mineralisation over CO_2 (Yu and Patrick, 2003).

4.2 Pathways of N transformation

The primary production mechanism of N_2O in the anoxic soil environment is denitrification. The intermediate products of denitrification were detected following the reduction of ^{15}N labelled NO_3^- (Fig. 3). The ^{15}N not accounted for 14-26 hrs after flooding is expected to be found as NO_2^- or NO . The recovery of ^{15}N was quantitative in treatment TN with effectively 100% of ^{15}N recovered as N_2 after 72 hrs. However, this was not the case for treatment TLN where after 144 hrs only 26% of the ^{15}N was recovered as N_2 with total ^{15}N recovery of $54 \pm 5\%$. Reasons for the lower recovery in TLN may be due to volatilization of NH_4^+ to NH_3 enhanced by the higher pH (Sommer and Hutchings, 2001), as the NH_4^+ concentration was lower in treatment TLN compared to TN (see SI Fig. S9) or that the residual ^{15}N was incorporated in other non-measured N pools. Alternatively, a major difference between the TN and TLN treatments at the end of the 144 hours was the higher NO_3^- concentration in the TLN treatment. Thus if any NO_3^- was lost as the soil cores were drained prior to performing

the KCl extracts then this may have had a greater impact on the ^{15}N balance in the TLN treatment. Another pathway may contribute to N_2O production. Only a small fraction of the applied ^{15}N was recovered as NH_4^+ for both treatments (Fig. 3). Although small, the increase over time indicates that NH_4^+ has been produced from DNRA of the applied $^{15}\text{N}\text{-NO}_3^-$ (Buresh and Patrick, 1978). The NH_4^+ produced from the applied $^{15}\text{N}\text{-NO}_3^-$ was minimal when compared to the total NH_4^+ concentration during the flooding event (see SI Fig. S9), but the process could potentially be important if flooding continued for prolonged periods. It also cannot be dismissed that the increase in $^{15}\text{N}\text{-NH}_4^+$ was caused by a release of assimilatory reduced $^{15}\text{N}\text{-NO}_3^-$ during extraction, even though the extraction setup should not destroy microbial cells.

4.3 N_2O emissions and agricultural management practice under future climate conditions

Based on these results, a marked reduction in N_2O production and emission during soil flooding could be achieved if soils were limed prior to tillage. As the reduction is achieved with only an increase in pH of 1.3 units it emphasises the importance of liming often, to keep the pH constant. Low lying areas are of highest risk of flooding and are also likely to receive additional inputs of N from the surrounding elevated areas. To reduce the risk of flood-induced N_2O emissions, N-application in low lying areas should be minimized and where possible these areas should be drained. If flooded, drainage should be avoided, as it is at the boundary between aerobic and anaerobic conditions when N_2O accumulation is seen. Consequently, there is a need for additional experiments to include more soil types and land uses before implications are scaled to larger areas.

438 **5. Acknowledgement**

439 The authors wish to thank Annie Wejhe Simonsen for performing the validation of the N₂O
440 diffusion results by INNOVA chambers and to thank the two anonymous journal reviewers
441 for helpful comments,

6. References

- Badr, O., Probert, S.D., 1993. Environmental impacts of atmospheric nitrous oxide. *Applied Energy* 44, 197-231.
- Berg, P., Risgaard-Petersen, N., Rysgaard, S., 1998. Interpretation of measured concentration profiles in sediment pore water. *Limnology and Oceanography* 43, 1500-1510.
- Brooks, P.D., Stark, J.M., McInteer, B.B., Preston, T., 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. *Soil Science Society of America Journal* 53, 1707-1711.
- Buresh, R.J., Patrick, W.H.Jr., 1978. Nitrate reduction to ammonium in anaerobic soils. *Soil Science Society of America Journal* 42, 913-918.
- Cappelen, J., Jensen, J.J., 2001. Jordens klima – Guide til vejr og klima i 156 lande (update). Teknisk rapport 01.07. Danmarks Meteorologiske Institut, Trafikministeriet, 191-193.
- Castellano, M.J., Schmidt, J.P., Kaye, J.P., Walker, C., Graham, C.B., Lin, H., Dell, C.J., 2010. Hydrological and biogeochemical controls on the timing and magnitude of nitrous oxide flux across an agricultural landscape. *Global Change Biology* 16, 2711-2720.
- Choudhary, M.A., Akramkhanov, A., Saggar, S., 2001. Nitrous oxide emissions in soils cropped with maize under long-term tillage and under permanent pasture in New Zealand. *Soil & Tillage Research* 62, 61-71.
- Clough, T.J., Sherlock, R.R., Kelliher, F.M., 2003. Can liming mitigate N₂O fluxes from a urine-amended soil? *Australian Journal of Soil Research*, 41, 439-457.
- Clough, T.J., Sherlock, R.R., Rolston, D.E., 2005. A review of the movement and fate of N₂O in the subsoil. *Nutrient Cycling in Agroecosystems* 72, 3-11.

464 Elberling, B., Damgaard, L.R., 2001. Microscale measurements of oxygen diffusion and
 465 consumption in subaqueous sulphide tailings. *Geochimica et Cosmochimica Acta* 65, 1897-
 466 1905.

467 Eriksen, J., Jensen, L.S. 2001. Soil respiration, nitrogen mineralization and uptake in barley
 468 following cultivation of grazed grasslands. *Biology & Fertility of Soils* 33, 139-145.

469 Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive
 470 measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology &*
 471 *Biochemistry* 35, 1231-1243.

472 Gundersen, J.K., Jørgensen, B.B., 1990. Microstructure of diffusive boundary layers and the
 473 oxygen uptake of the sea floor. *Nature* 345, 604-607.

474 IPCC, 2007. *Climate Change 2007: The Physical Science Basis. Contribution of Working*
 475 *Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.*
 476 [Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller,
 477 H.L. (eds)]. Cambridge University Press.

478 Jørgensen, C.J., Elberling, B., 2012. Effects of flooding-induced N₂O production,
 479 consumption and emission dynamics on the annual N₂O emission budget in wetland soil. *Soil*
 480 *Biology & Biochemistry* 53, 9-17.

481 Knowles, R., 1982. Denitrification. *Microbiological Reviews* 43-70.

482 Leff, B., Ramankutty, N., Foley, J.A., 2004. Geographic distribution of major crops across the
 483 world. *Global Biogeochemical Cycles* 18, GB1009.

484 Le Mer, J., Roger, P., 2001. Production, oxidation, emission and consumption of methane by
 485 soils: A review. *European Journal of Soil Science* 37, 25-50.

486 Liengaard, L., Nielsen, L.P., Revsbech, N.P., Priemé, A., Elberling, B., Enrich-Prast, A.,
 487 Köhl, M., 2013. Extreme emission of N₂O from tropical wetland soil (Pantanal, South
 488 America). *Frontiers in Microbiology* 3, 1-13.

489 Min, S.-K., Zhang, X., Zwiers, F.W., Hegerl, G.C., 2011. Human contribution to more-intense
 490 precipitation extremes. *Nature* 470, 378-381.

491 Molloy, L., 1988. Soils in the New Zealand Landscape, the living mantle. *Mallinson Rendel*
 492 in association with the New Zealand Society of Soil Science, 179-181.

493 Moraghan, J.T., Buresh, R., 1977. Correction for dissolved nitrous oxide in nitrogen studies.
 494 *Soil Science Society of America Journal* 41, 1201-1202.

495 Mosier, A.R., Mack, L., 1980. Gas chromatographic system for precise, rapid analysis of
 496 nitrous oxide. *Soil Science Society of America Journal* 44, 1121-1123.

497 Mulvaney, R.L., Boast, C.W., 1986. Equations for determination of nitrogen-15 labeled
 498 dinitrogen and nitrous oxide by mass spectrometry. *Soil Science Society of America Journal*
 499 50, 360-363.

500 Pan, Y., Ye, L., Ni, B.-J., Yan, Z. 2012. Effect of pH on N₂O reduction and accumulation
 501 during denitrification by methanol utilizing denitrifiers. *Water Research* 46, 4832-4840.

502 Potter, P., Ramankutty, N., Bennett, E.M., Donner, S.D., 2010. Characterizing the spatial
 503 patterns of global fertilizer application and manure production. *Earth Interactions* 14, 1-22.

504 Ramsing, N., Gundersen, J., 2009. Seawater and Gases. Tabulated physical parameters of
 505 interest to people working with microsensors in marine systems. Unisense.

506 Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous oxide (N₂O): The dominant
 507 ozone-depleting substance emitted in the 21st century. *Science* 326, 123-125.

508 Roelandt, C., Van Wesemael, B., Rounsevell, M., 2005. Estimating annual N₂O emissions
 509 from agricultural soils in temperate climate. *Global Change Biology* 11, 1701-1711.

510 Silgram, M., Shepherd, M.A., 1999. The effects of cultivation on soil nitrogen mineralization.
 511 *Advances in Agronomy* 65, 267-307.

512 Šimek, M., Cooper, J.E., 2002. The influence of soil pH on denitrification: progress towards
 513 the understanding of this interaction over the last 50 years. *European Journal of Soil Science*
 514 53, 345-354.

515 Sommer, S.G., Hutchings, N.J., 2001. Ammonia emission from field applied manure and its
 516 reduction – invited paper. *European Journal of Agronomy* 15, 1-15.

517 Stevens, R.J., Laughlin, R.J., Atkins, G.J., Prosser, S.J., 1993. Automated determination of
 518 nitrogen-15 labelled dinitrogen and nitrous oxide by mass spectrometry. *Soil Science Society*
 519 *of America Journal* 57, 981-988.

520 Tiedje, J.M., Sexstone, A.J., Myrold, D.D., Robinson, J.A., 1982. Denitrification: ecological
 521 niches, competition and survival. *Antonie van Leeuwenhoek* 48, 569-583.

522 Weier, K.L., Doran, J.W., Power, J.F., Walters, D.T., 1993. Denitrification and the
 523 dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil*
 524 *Science Society of America Journal* 57, 66-72.

525 Wrage, N., Velthof, G.L., van Beusichem, M.L., Oenema, O., 2001. Role of nitrifier
 526 denitrification in the production of nitrous oxide. *Soil Biology & Biochemistry* 33, 1723-
 527 1732.

528 Yu, K., Patrick, W.H.Jr., 2003. Redox range with minimum nitrous oxide and methane
 529 production in a rice soil under different pH. *Soil Science Society of America Journal* 67,
 530 1952-1958.

531 Zárte-Valdez, J.L., Zasosiki, R.J., Läuchli, A.E., 2006. Short-term effects of moisture
532 content on soil solution pH and soil Eh. Soil Science 171, 423-431.

533 **Supporting information**

534 Figure S1: Distribution of KCl extractions over time.

535 Figure S2: Timing of repeated measurements.

536 Figure S3: Validation of microsensor measurements by chamber measurements.

537 Figure S4: Maximum N₂O concentration over time for t₁, t₂ and t₃.

538 Figure S5: N₂O concentration, diffusion flux, redox potential and NO₃⁻ concentration
539 development over time.

540 Figure S6: Relationship between N₂O concentration and redox potential.

541 Figure S7: Depth-specific activity of N₂O production and consumption.

542 Figure S8: Atom% ¹⁵N enrichment.

543 Figure S9: Concentration of NO₃⁻, NH₄⁺, CH₄ and the total sum of N over time.

544 **Table 1.** Overview of soil pH, NO_3^- and DOC concentration as well as the absolute and the
545 primary redox range and the redox range for the ‘build-up’ and ‘build-down’ phase of N_2O
546 for all four treatments (n =3).

	Control (TC)	Limed (TL)	N added (TN)	N added & limed (TLN)
<i>pH</i>	6.1 ± 0.1	7.4 ± 0.1		
<i>NO_3^- ($\mu\text{gN g soil}^{-1}$)</i>	97.8 ± 27.6	108.9 ± 49.9	197.9 ± 73.3	183.3 ± 31.8
<i>DOC ($\mu\text{g g soil}^{-1}$)</i>	129.4 ± 3.9	160.9 ± 2.7		
<i>Primary redox range (mV)</i>	300-450	200-350	250-450	300-400
<i>N_2O build-up redox range (mV)</i>	414-339	335-242	418-312	384-331
<i>N_2O build-down redox range (mV)</i>	366-289	298-194	342-232	361-282

547

Figure 1. Development of mean maximum N₂O concentration and observed diffusion flux (a), time- and depth-specific contour plot of the N₂O concentration over time for treatment TC (b), time- and depth-specific contour plot of the redox potential over time for treatment TC (c) and the sum of the NO₃⁻ concentration in the soil core over time (d). For contour plots of treatment TL, TN and TLN see SI (Fig. S5; 5A, 5B and 5C respectively).

Figure 2. Modelled activity of N₂O production (positive values) and consumption (negative values) over 6 time intervals (10, 20, 30, 50, 70 and 90 hrs after flooding) for treatment TC (solid line) and TN (dashed line). Values < 0.0007 mol cm⁻³ s⁻¹ are not shown.

Figure 3. ¹⁵N recovered as NO₃⁻, N₂O, N₂ and NH₄⁺ (secondary axis) over time for treatment TN (a) and TLN (b). All values are normalized against the recovered ¹⁵N-NO₃⁻ at time 0 (n = 3).